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The Journal of Laboratory and Clinical Medicine

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30th Annual Report

Physicians Casualty Association **Physicians Health Association**

Omaha, Nebraska

Balance Jan. 1, 1931 (Less $2,556.48 Depreciation on Furniture and Fixtures) $1,027,743.58

INCOME

Collected from members	$638,232.02	
Net Interest	47,046.04	
Miscellaneous	202.37	685,480.43
		$1,713,224.01

DISBURSEMENTS

Sick and Accident Claims paid	$528,007.83	
All other expenditures...	101,077.04	629,084.87
Balance Dec. 31, 1931		$1,084,139.14

ASSETS

Cash (First National Bank, Omaha)	$ 49,139.66	
Bonds	872,093.97	
Real Estate Mortgages..	121,862.50	
Real Estate	30,308.41	
Furniture and Fixtures..	10,200.00	
Collection Items	534.60	$1,084,139.14

Purely Mutual
No Agents
No Profits

Danger of Restricted Diets

Often Low In Vitamin-B (F & G) Causing Secondary Effects

In the Jour. A. M. A., Nov. 14, 1931, Dr. Russell M. Wilder, Chicago, says:

> "I should like to mention two cases of pellagra that developed in patients that were under a dietary treatment for epilepsy.
> "In the two cases referred to—the addition of Brewers' Yeast alone, without other alteration of the diet, promptly resulted in a cure. Since then I have made a practice of including yeast in all my epileptic diets and thus far have not again encountered this complication."

Likewise, the "restricted diets" of diabetes, obesity and the like, often reduce the content of vitamine-B (F & G) to a dangerously low level.

Dr. H. C. Sherman (Jour. A. M. A., Nov. 14, 1931) says:

> ". . . so a liberal intake of vitamine-G contributes to a better than average nutritional condition and thus to what McCollum and Simmonds have aptly termed 'the preservation of the characteristics of youth'."

BREWERS' YEAST-HARRIS
(DRY POWDER)

has been adopted by the U. S. Public Health Service for the successful treatment of pellagra of the south. It is indicated in the following conditions:

Accessory to deficient diets
Anemias
Convalescence
Immunity Building

Yeast Vitamine-Harris Tablets are made from a concentrate of this same yeast. They are smaller in volume, palatable, contain a known amount of vitamine-B, and are used by physicians where smaller dosage is required.

Samples on Request by

The Harris Laboratories, Inc.
Tuckahoe, New York

The Journal of Laboratory and Clinical Medicine

VOL. XVII ST. LOUIS, MO., MARCH, 1932 NO. 6

SYMPOSIUM ON CLINICAL BACTERIOLOGY

THE BROADER ASPECTS OF ROUTINE CULTURAL EXAMINATIONS*

BY RUTH GILBERT, M.D., ALBANY, N. Y.

THE subject is so comprehensive that the discussion is confined to a résumé of factors which tend to enhance the value of those aspects of bacteriologic procedures employed in examinations for physicians and sanitarians. Experience has proved that the work should be undertaken only in case it can be done expertly. The physician had better trust to his clinical findings and the sanitarian depend upon his inspection of environment or of methods and equipment than attempt to secure data from a laboratory unless practically implicit reliance can be placed upon its reports. The character, education, and training of the director and his assistants are thus of primary importance. The nature of the training which is required depends somewhat on the kind of work undertaken. If information is desired which would be of assistance in the diagnosis or treatment of individuals who are ill, the person in charge, besides having had adequate training and experience in pathology and bacteriology and in the fundamentals of chemistry, should be familiar with the clinical manifestations of disease. In case the laboratory serves the sanitarian also, its director should, in addition, have a knowledge of pertinent phases of sanitary engineering and the principles underlying the production of the materials to be tested. The technical assistants needed for all types of bacteriologic work should be thoroughly reliable, and keen to note and report to their superior any unusual results which may be obtained. Their training should be adequate for the procedures assigned to them. Unsterile equipment and faulty technic may of course entirely vitiate the significance of bacteriologic findings.

A sufficient amount of satisfactory equipment is important. While an ingenious bacteriologist can often improvise suitable apparatus, his time and energy should be conserved for his professional duties if the supplies needed are purchaseable.

*From the Division of Laboratories and Research, New York State Department of Health, Albany.

The procedures followed in bacteriologic examinations depend somewhat on the purpose for which they are undertaken; that is, whether they are designed to aid in diagnosis or to determine whether the patient is a carrier of a certain species of bacteria or can safely be released from quarantine. Another distinct group of examinations consists of those made for purposes of controlling sanitary conditions in the environment.

In case the physician wishes aid in diagnosis, the laboratory worker should, wherever possible, study the patient with the clinician and arrange for the collection of suitable material for examination. When this direct contact with the patient cannot occur, the physician submitting the specimen should furnish all the pertinent data which are available. The bacteriologist is then in a position to render intelligent service. When tests are undertaken to demonstrate evidence of the carrier condition or in connection with release from quarantine, examinations of a routine nature are usually all that are required, although proper precautions must be taken to insure the collection of suitable specimens. Samples of water should be accompanied by information concerning the sanitary conditions which prevail in the environment of the sources; otherwise, the results of bacteriologic tests cannot be evaluated.

It is most desirable that samples of milk and water be examined in accordance with an established routine (standard methods), so that results from all laboratories may be comparable. Dairymen and other producers who have difficulties in maintaining their products at an adequate sanitary standard can frequently be given material assistance through the interpretation of some of these findings. The species of bacteria present often suggest their source and the points at which defects or faulty methods may be expected.

Specimens examined for purposes of diagnosis do not lend themselves to a definite routine. Each one must be considered individually, and only as every case becomes a research problem will the best type of service be rendered. In addition to the clinical manifestations, the results of other tests or studies of his metabolism, tissue, blood, body fluids, or discharges must be considered. In fact, all of the data elicited during the examination of the patient should be correlated with the bacteriologic findings. At times, a simple morphologic examination of exudate may be sufficient to confirm the diagnosis, as in the case of gonorrhea and epidemic meningitis. More frequently, however, the bacteria seen in preparations from lesions do not have a sufficiently characteristic appearance to permit their identification, or microorganisms may not be found in such preparations. The history of the case may then provide a basis for determining the type of cultural study to be undertaken, the medium which will probably be most suitable, and whether aerobic or anaerobic conditions should be provided. When there is any uncertainty, experience has shown the desirability of inoculating sufficient medium to permit part of it being kept under aerobic, another portion under anaerobic, conditions, and the remainder in an atmosphere containing from 5 to 10 per cent of carbon dioxide.

The microscopic examination of the original material, exudate for example, which is too often omitted, is essential to determine the relative number and kinds of bacteria, and finally to interpret the significance of the results of cultural study. The morphologic findings and knowledge of the possibilities for

contamination of the specimen during collection, indicate the amount of dilution required and the necessity for employing differential media or other means for the isolation of significant species which may be present.

The time of incubation is most important. A long period may be necessary to secure growth. This is most frequently true in the case of highly parasitic species found in chronic infections, such as tuberculosis, certain streptococcus infections, etc. A month is none too long for the incubation of blood cultures, if growth is not secured earlier. Animal inoculation furnishes another means for determining the presence of some of the pathogenic bacteria. The species of animal chosen and the method of inoculation depend largely on the history of the case and the result of the morphologic examination of the specimen.

When cultures of microorganisms have been secured, identification usually requires a study of their morphology, biochemical reactions, behavior in sera from immunized animals, and of the type of lesion produced in inoculated animals. The evaluation of the findings may require a consideration of all the information secured in the study of the case. At times it may be desirable to determine whether the patient shows evidence of hypersensitivity to products of the bacteria which were isolated, or whether significant reactions can be secured with them in a specimen of his blood serum. The possibility of mixed infections must always be kept in mind. Either syphilis or tuberculosis, or both of these conditions, may of course complicate almost any other infection. When there is any doubt concerning the etiologic significance of the bacteriologic findings, specimens for confirmatory examination should, whenever possible, be secured.

To summarize, bacteriologic examinations made for purposes of conserving the public health, such as sanitary examinations of milk and water samples and those tests performed before release from quarantine or to determine the carrier condition, can well be conducted in accordance with standardized methods. Laboratory work which serves as an aid in diagnosis must, on the contrary, be undertaken, in most cases, as a special investigation, the procedures being chosen in the light of all the information available from a careful study of the patient.

USE OF ANIMALS IN ROUTINE DIAGNOSTIC WORK*

By K. F. Meyer, Ph.D., San Francisco, Calif.

LONG before the animate nature of disease was conclusively established Henle formulated a statement of the prerequisites which must be fulfilled in order to prove that a particular agent was the cause of an infective malady. He maintained that the agent must constantly be found in the disease. This constant agent must be isolated and tested in the isolated state to note whether it is capable of producing the disease. Obviously, this statement contains all the elements quite generally but incorrectly referred to as the "postulates of Koch (1878)." They serve as the unassailable basis upon which all the work on specific pathogenic causes is built. Despite the fact that subsequent observations placed certain restrictions and modifications on the original proposition, the testing of the isolated object or agent of disease must always remain a function of the bacteriologist. It is to be regretted greatly that the guiding propositions of Henle have frequently been overlooked in the routine diagnostic work of the modern laboratory. The reasons for this neglect are probably twofold: (1) Many of the workers who are called upon to do diagnostic work have had little or no experience in pathology. Even those who attend university courses have rarely had the opportunity to test the disease-producing properties of the common microorganism which they have isolated and painstakingly studied in the test tube. Lack of adequate funds, an unpardonable disinterestedness or one-sided training of the instructors may be mentioned as additional factors. (2) Though the importance of the pathogenicity tests in bacteriologic work is admitted, until recently little attention has been paid to the animals which are required for the examinations. Only well-equipped diagnostic laboratories feel justified in maintaining an adequate stock of healthy animals. In some laboratories the number of rabbits and guinea pigs is so limited that each animal is a pet readily identified by an endearing name instead of a number. Others doubtless exist in which the animals are kept under unsanitary conditions and without proper care and feeding. Avitaminoses and chronic or intercurrent diseases are prevalent. It is needless to emphasize that costly, unpleasant surprises and many misleading observations are the obvious corollaries to such a state of affairs. Probably other criticisms could be offered. Suffice it to state that the prevailing conditions are largely the result of our own neglect. Until recently few publications paid attention to the "living test tubes" or the animals, largely because animal experimentation was considered the realm of the specialist and the study of the maladies of man a field which could not profit by an inquiry into the diseases of animals. More and more has this narrow conception vanished and the recognition that some communicable diseases of laboratory animals furnish excellent material for the

*From the George Williams Hooper Foundation, University of California, San Francisco, California.

study of fundamental problems in pathology promises an entirely new orientation.

It is obviously not the purpose of this article to outline the various trends but merely to indicate the newer knowledge which the diagnostician must keep constantly in mind when he works with animals. In general, the use of animals in the diagnostic laboratory is confined to the following procedures: (1) As a culture medium for the detection or rapid cultivation of various microorganisms. (2) For the purification or elective isolation of certain types of bacteria from mixtures. (3) For the propagation of virus diseases which cannot be maintained in artificial cultures. (4) For the study of the pathogenicity or virulence and the pharmacologic and toxicologic effect of metabolic products. (5) For the preparation of reagents, such as complement or diagnostic sera. (6) To test the harmlessness or other effects of biologic products as well as their sterility, as in the case of filterable viruses.

With unremitting insistence, it must be emphasized that the execution of these procedures is successful only when certain fundamental principles are carefully considered and continuously carried out. The animals must be healthy, preferably selected from a stock with a known hereditary history. They must be housed under sanitary conditions and fed an appropriate, well-balanced diet. The selection of an ideal place for the breeding of the required stock of guinea pigs and rabbits offers many technical difficulties. Adequate stable facilities, properly trained personnel, and an abundant supply of inexpensive food are not usually at the disposal of the majority of diagnostic laboratories, or when available, require an overhead expenditure which is entirely out of proportion to the number of animals required. For these reasons, the laboratories supply their demands by random purchases from dealers or breeders. The inherent disadvantages of such a policy are numerous. Rarely, if ever, can the local market supply the varying demands for animals of different age, color, weight, sex, etc. Suitable animals in emergency for unexpected tests may therefore not always be in stock and the dealer supplies animals quickly assembled from various breeders. Such shipments may contain diseased animals. Until each city or state has a central animal supply station, it is advisable to restrict the purchase of guinea pigs, rabbits, and mice to that of contract from reliable dealers who raise their own stock and refuse to supply any other animals. Even these precautions fail only too frequently and expose the laboratory to the risk of introducing animals infected with a communicable disease. In order to reduce the danger it is imperative to hold each shipment in quarantine for at least two weeks. A special room should be reserved for this purpose and the animals should be housed in small groups. Preferably a single rabbit, with pairs or lots of four guinea pigs, may be placed in the same cage. The tag on the cage should carry a detailed history of the shipment. The results of the daily inspections should be recorded either on the tag or in a special book. Provocative tests for snuffles or stool examinations for Salmonella organisms and parasites should be carried out. Animals that die should be carefully autopsied and such laboratory examinations instituted as may be deemed necessary. The data should be submitted either to the chief animal caretaker or to the person responsible for the animal quarters. In case an infectious

disease is discovered it is much more economical to use the shipment for sacrifice experiments than to prolong the quarantine and trust to luck that the malady will not spread. Latent infections with the B. cuniculisepticus are frequently activated by the injection of biologic products and thus the experimental animals may act as spreaders of the disease. It appears unnecessary to emphasize that the caretaker in charge of the quarantine room should exercise special care in the disinfection of the feeding cups, cages, etc., and be instructed in methods to break the chain of transmission through utensils, hands, or clothing, from the quarantined stock to the experimental animals. While in quarantine the animals are freed from vermin and ectoparasites. These things appear self-explanatory but they are often overlooked.

The suggestion that a group of laboratories in a state or city pool their resources and encourage an interested party to breed laboratory animals for certain purposes deserves serious consideration. More and more it becomes evident that the fundamental laws of infection must be studied on litter mates or preferably on animals with a known hereditary history. The brilliant researches of Maude Slye on the transmissibility of tumors, those of Lewis and Wright on the varying susceptibility of guinea pigs to tuberculosis, and those of Webster and Pritchett on the inborn, nonspecific resistance of mice against B. aertrycke infection offer ample proof that in the future even diagnostic studies must be conducted on animal species with a known inherent susceptibility to certain infections. Provided the animal breeding institute is under the supervision of a geneticist, an abundance of invaluable information can be collected and subsequently correlated with the findings of the biologist and pathologist. Thus, the need for special, short-haired rabbits or albinos for skin tests could be met with ease, and litter experiments would be more frequently conducted than has hitherto been the case.

Those who desire to raise a limited stock of mice or guinea pigs should familiarize themselves with the behavior, breeding habits, and gestation periods. Excellent, thoroughly scientific summaries on the mouse, rat, guinea pig and rabbit are available in book form (Keeler, Donaldson, Raebiger) or in a chapter of the *Handbuch der pathogenen Microorganism* and in *A System of Bacteriology in Relation to Medicine.*

There is an increasing tendency to standardize the *cage equipment* in the different laboratories. Excellent designs, although expensive, are now manufactured by several companies. Metal cages are quite generally used for individual experimental animals, while wooden runs or entire rooms with wooden floors are preferred for stock guinea pigs and rabbits. However, it is well to remember that even the best cages will not prevent disappointment when their care is superficial and sporadic. It appears unnecessary to outline the essentials since Wadsworth has admirably summarized the rules which should be followed. Cages or jars which house animals with highly communicable diseases should be kept in special rooms and should only be handled by the worker in charge of the respective tests. In fact, he or she should be directly responsible for the feeding of the animals while in use, and the disinfection and cleaning of the containers after they are vacated. It is doubtless an advantage to tag such cases or jars with specially colored labels. Many laboratories use sawdust as

a bedding. Experience has taught that shavings are preferable, particularly for rats. It is not unlikely that the troublesome lung disease of these animals is greatly augmented by improper bedding.

The animal quarters should be well lighted and ventilated. A uniform temperature during the entire day and night should be provided. Special attention must be given to the feeding of the animals. Only too often this phase of the animal experiment is sadly neglected. Although excellent diet tests have been reported, it appears desirable for quick reference to summarize the pertinent data with respect to each species of animals:

1. *Mice* may receive stale bread soaked in water or skimmed milk. A small amount of cod liver oil may be added once a week (approximately one ounce for 300 mice). Crushed barley or rolled oats or moistened middling may be offered at regular intervals. An excellent ration recommended by Keeler consists of rolled oats (240 parts), powdered skim milk (30 parts), cod liver oil (8 parts), and salt (one part). The formula for rat feed prepared by Maynard (*Science* 71: 192, 1930) is equally satisfactory. Greens in the form of lettuce or clover should be given occasionally. Drinking water must be available always.

2. *Rats.*—One can either use the Maynard standard diet or prepare a mash which consists of boiled beans, wheat maize meal, cabbage, and cod liver oil. Beef or liver and fresh cabbage should be offered once a week. A mixture of boiled vegetables supplemented by oats, corn, or white bread mixed with milk is equally satisfactory. Drinking water should always be available.

3. *Guinea Pigs and Rabbits.*—Rolled oats or crushed barley, bran, and a good quality of alfalfa or clover hay represent the basic ration which must be supplemented with green feed, either cabbage or carrots, and beets. From time to time, salt, fish meal, and boiled potatoes may be offered. If the food contains considerable moisture, no water need be supplied. Spontaneous scurvy is by no means uncommon among guinea pigs maintained on a diet which lacks green stuffs (Smith, Holst and Frölich, Funk).

Aside from a careful selection of freshly prepared food mixtures, or wholesome vegetables and greens freed from tainted or rotten spots, it is important that a regular system of feeding be strictly adhered to. All animals should be fed and cared for once a day, including Sunday. A double ration of food thrown into the cages on Saturday will not supply the required nourishment on Sunday.

A dependable system of identification of the animals should be adopted. The practice of cage labeling is suitable for stock animals kept in large groups, although in the interest of complete records each animal should be numbered and registered with regard to sex, description, etc. Guinea pigs and rabbits may be identified readily with the aid of aluminum ear tags (Aluminum Marker Works, Beaver Falls, Pa.) or India ink ear marking sometimes supplemented by a diagrammatic notation in the record book, while rats and mice are marked either by puncturing the ears, a system introduced by the American geneticists, or by color markings with aniline dyes. In the diagnostic laboratory, the latter procedure recommends itself on account of its simplicity.

The worker who uses animals should be thoroughly familiar with the normal anatomy and physiology of the guinea pig, rabbit, rat, and mouse. He

should have at his disposal such standard works as W. Schauder, *Anatomie der Impfsaugetiere* and in particular the monumental treatise by Rudolf Jaffé, *Anatomie und Pathologie der Spontanerkrankungen der Kleinen Laboratoriumstiere* and the *Tabulae biologicae*. Valuable information will also be found in the well-known treatises on the rabbit by Bensley and on the rat by Hunt.

Systematic postmortem examinations of every animal which is found dead in the animal house or those which are sacrificed for various reasons offer an inexhaustible source of information and training. Freedom from epidemic or parasitic diseases which, when not recognized, may seriously invalidate an animal test, largely depends upon these thorough and conscientious anatomic examinations, as well as upon an appreciation of the epizootiology of the infections and their effective control by quarantine and the other preventive measures. Without a fundamental knowledge of the normal and pathologic anatomy of the common laboratory animals the diagnostician and research worker fail to appreciate the limitation of the animal tests. The significance of spontaneous infections and parasitic invasions in the animal stock is now fully recognized and in addition to the book by Jaffé, several articles (K. F. Meyer, Raebiger and Lerche, Remlinger and P. Bell, O. Seifried, Gerlach, Farmer's Bulletin No. 1568) have detailed the most important facts. It is not within the scope of this review to attempt even a brief summary of the many maladies which may be encountered. An opportunity will be afforded to illustrate the importance of the intercurrent disease as factors in the diagnostic animal test.

Every modern textbook of bacteriologic technic (Kolmer and Boerner, Smith, Eyre, Wadsworth, Haberland, etc.) contains excellent commentaries on the methods of manipulation, inoculation, and bleeding of animals, but one essential principle is rarely stressed. Immediately upon the inoculation of animals a period of clinical observation begins which should terminate only with the death of the animal. These observations should always take cognizance of:

(a) *The General Appearance.*—Inspection once or preferably twice daily should be given with a view to detect symptoms. Each animal should be observed in motion; the consumption of food and water and the appearance of the

TABLE I

NORMAL TEMPERATURE, PULSE, AND RESPIRATION OF EXPERIMENTAL ANIMALS

ANIMAL	AVERAGE RECTAL TEMPERATURE °C. AND °F.	PULSE RATE	RESPIRATION PER MINUTE
Guinea pig	38.6° C.; 39.4° C.—7.5 cm. from anus (101.5° F.—4 cm. from anus) (Minimum 37.8° C., maximum 40.5° C.)	150	100-150
Rabbit	39.6° C. (102.4° F.) (Minimum 38.3° C., maximum 40.8° C.) No temperature under 40.0° C. should be considered pathologic	120-140	50-60
Rat	37.9° C. (100.0° F.)	—	210
Mouse	37.4° C. (99.3° F.)	120	—

feces should be noted. Attention should also be paid to possible salivation, nasal and conjunctival discharges, and to the reactions at the site of inoculation.

(b) The *weight* should be recorded before experiment and afterwards at weekly intervals or more often as the circumstances require. Each weighing should be done as nearly as possible under the same conditions as the first one.

(c) The *temperature* should be taken in many cases before beginning the particular experiment and subsequently on each successive day at the same hour. For the sake of convenient reference the normal average temperature is given in Table I in order to prevent the erroneous assumption that a pyrexia is present in an animal which shows merely its own normal temperature.

(d) *A hematologic study* is frequently indicated. The general principles are the same as customarily used in the diagnostic laboratory. The normal averages of the different blood cells are summarized in the book by Klieneberger and W. Carl, and in the chapters by E. Haam and E. Flaum in the Handbuch by Jaffé (p. 155).

(e) *The bacteriologic, serologic, and autopsy* examinations differ in no way from those generally employed.

Provided the principles briefly outlined are followed the diagnostician should experience no disappointment in the use of the animal test. Since a perusal of the usual text fails, however, to mention the type of animal most suited for certain examinations, it is deemed advisable to offer certain suggestions. Thus, an opportunity will be afforded to indicate the complications which may arise should the mice, guinea pig, or rabbit be spontaneously diseased. For the sake of convenience, the subject is discussed under the heading of the respective infections:

1. *Staphylococcus Infections of Intoxications.*—The pathogenicity of a staphylococcus should be studied on the rabbit or on Japanese white mice. Significant lesions may be observed following intravenous injections, while local reactions may be induced by scarification of the cornea. Toxic metabolic products may be tested by intravenous injection (Dack and associates) or by intradermal application (Parker, 1924).

2. *Streptococci.*—There is some difference of opinion as to which animal is most susceptible. If relative sizes of animals are considered, the rabbit is the most readily attacked. Various methods of infection are chosen in order to imitate the pathologic process from which the coccus has been isolated. Virulence tests are frequently made on mice. Great care should be exercised in the interpretations of the bacteriologic findings on these animals since several workers (Zlatogoroff, Palanto and Kochkine, Grossmann and others) have found that hemolytic and nonhemolytic streptococci have been isolated from supposedly "normal mice." Therefore, the supposed mutation of pneumococci and nonhemolytic varieties by intraperitoneal injections (Morgenroth, Schnitzer and Berger) deserves further investigation. Latent infections due to hemolytic streptococci are quite common in guinea pigs. They frequently invalidate the diagnostic experiments.

3. *Pneumococcus Infections.*—The extreme susceptibility of the mouse to the pneumococcus is a commonplace of laboratory experience and forms the

basis for the rapid isolation of the organism from bacterial mixtures, sputum, etc. Although the mouse may be killed by a smaller dose than the rabbit, it is generally recognized through the comparative studies of Cafeiro, Cotoni, Truche and Raphael that the rabbit is more susceptible. In an emergency this animal can therefore be used for the typing of pneumococcus strains. Experimentation on guinea pigs requires careful and critical interpretations since spontaneous pneumococcal infections may be unusually common in the stock of certain breeders.

4 *Influenza Bacilli.*—It is well to remember that autolyzed influenza bacilli may set up spontaneous streptococcal or pneumococcal infection in guinea pigs and mice. A general blood infection may be produced regularly when the sputum to be tested contains the symbiotic adjuvants, the cocci (Wolf, 1920, Huntoon and Hammin).

5. *Tuberculosis.*—The detection of tubercle bacilli by animal inoculation offers several disadvantages. Not infrequently this species suffers from chronic diseases, such as pseudotuberculosis or Salmonella infections which present gross anatomic lesions readily confused with those of tuberculosis. Spontaneous tuberculous infections of the guinea pig and rabbit have been reported by Feyerabend, Seifert, Sewall and Lurie, Stanley Griffith and others, and thus may greatly invalidate the significance of the test. In part, these disadvantages may be overcome by a judicious interpretation of the postmortem findings. It is a well-known fact that in susceptible animals the primary localization of the lesion always indicates the avenue of infection. If, for example, after subcutaneous inoculation a tuberculosis of the tracheobronchial lymph nodes with no disease in the inguinal nodes is found, one may conclude that the infection was acquired from extraneous sources.

6. *Plague.*—*B.* pseudotuberculosis rodentium (Pfeiffer) occurs spontaneously in guinea pigs and may thus interfere with the diagnosis of plague (Indian Plague Commission, J. Hyg. 7: 256, 1907).

7. *Brucella Infections.*—It is not generally appreciated that isolated cases of melitensis (Nicolle and Conseil) and abortus infections (Surface) have been reported in animals. A extensive epizootic which was caused by a melitensis type and affected 400 guinea pigs has been reported by Zdrodowski (1927 and 1930). In view of the wide distribution of the Brucella organisms, it is imperative that in the future shipments of guinea pigs from unknown breeders be scrutinized for Brucella infection. Agglutination tests previous to the inoculation of the test material are exceedingly useful.

8. *Anaerobic Infections.*—Those who use the guinea pigs or rabbits for the primary isolation of a pathogenic anaerobe should always remember that certain species may be found as common parasites in the intestinal tube or even in the organs of these animals (Heller, E. Schmidt).

9. *Paratyphoid Infections.*—The fact that B. aertrycke orally administered to mice sets up a lethal septicemia, while B. paratyphosus B (Schotmüller) usually does not, is sometimes used as a practical differential test of these two organisms. Unfortunately, its value is greatly reduced on account of the widespread occurrence of latent aertrycke infection in the rodents. Experimentation

with paratyphoid bacilli on mice requires sound judgment. The bacteriologic literature reports such bizarre findings as the transformation of a B. paratyphosus into a B. enteritidis or B. aertrycke.

10. *Virus Infections.*—The controversial literature on experimental encephalitis clearly shows that latent widespread parasitic infections may be responsible for misleading conclusions. Furthermore, the discovery of a new virus by Miller, Andrewes and Swift, in the course of an attempt to reproduce experimental rheumatic fever, furnishes another example of the many obstacles which may continue to render the animal test a very complicated diagnostic procedure.

Many more observations could be cited. In particular, attention might be drawn to the deficiency of the hemolytic complement in the blood of certain races of guinea pigs owing to the absence of the third component (Hyde). Suffice it to emphasize that the bacteriologist who employs the animal as a test object or model in his diagnostic work should be in a position to defend his claims that the lesions or findings are not those of a spontaneous disease but the result of the experiment. This aim can be met to a great extent if he employs only well-bred animals with a known hereditary history free from bacterial infections and parasitic invasions, maintained and cared for by an experienced personnel, and kept on well-balanced diets in a sanitary and hygienically controlled environment.

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ANAEROBIC TECHNIC*

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ATTENTION was directed by Pasteur (1859-61), during his investigations dealing with the socalled "butyric ferment," to the existence of microorganisms capable of living and multiplying in the absence of oxygen. He subsequently introduced the terms "aerobe" and "anaerobe" to indicate and emphasize the gaseous requirements of microbic life. As a result of the publications of Koch on anthrax, Pasteur became interested in this disease. With his associate, Joubert, suspected anthrax blood that had undergone putrefaction was studied and from this material an anaerobe, the vibrion septique, was isolated. Experiments demonstrated the new germ to be pathogenic for animals. Quite naturally this work stimulated the search for other germs which bore this peculiar relationship to air, with the result that several anaerobic organisms were early detected and technical methods for their cultivation were developed and employed.

From the time of Pasteur's discovery down to the present day, there has been one continuous stream of anaerobic apparatus of every size, shape, design, and material, all based on permutations and combinations of a few elementary principles; namely, exclusion of air, exhaustion of air, absorption of oxygen, replacement of air with an inert gas, apparent presence of air, microbic or tissue association. It is characteristic that each device or procedure is usually referred to as new and simple.

The anaerobic bacteria have ordinarily constituted a class by themselves, set apart from the usual routine in bacteriology, unquestionably because of the supposed difficulties, of a technical nature, attending their investigation. The problems surrounding their study have been exaggerated and overemphasized (perhaps unintentionally) by teachers, to the extent that there has been instilled in the mind of the ordinary laboratory worker the feeling that anaerobic life is a field of investigation reserved for those specially trained workers, of which each nation had but few, with an unlimited supply of fanciful equipment. In fact, during the past only a very few laboratory courses in bacteriology included actual experiments on the isolation and identification of anaerobes. That this class of organisms was of great economic importance was well known; not only were they associated with a number of diseases of man and animals but it was understood that putrefaction, or at least the initiation of putrefaction, was a process which these bacteria carried on almost alone.

The World War focused attention on anaerobic technic primarily because of the serious consequences resulting from the multiplication of these organisms in wounds with their sequel of gas gangrene. It is true that such infections had commanded attention before, but the incidence was rather infrequent.

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Commissions were appointed to search the literature for practical procedures; to develop methods if such were not in existence, and to test thoroughly all recommendations under routine laboratory conditions, with the end in view of making available a simple satisfactory technic for the study of this group. Adequate directions were found, which, if followed, gave excellent results in the hands of those willing to employ the precautions essential to insure the complete absence of air during the incubation period of the culture, as well as a minimum exposure of the germs to air at all times. It is deserving of mention that the quantity of oxygen which inhibits the growth of anaerobes is small.

The concentration by many workers on the flora of war infections resulted in the addition of several members to this group, and also brought together, in an easily available form, the literature relevant to the subject. But no striking technical procedures or new pieces of apparatus were evolved which demonstrated marked superiority to the methods of twenty years before. It is characteristic of the postwar literature, however, to refer to "the chaotic state of the pre-war anaerobic knowledge and the fundamental advances due to improved methods of anaerobic cultivation developed during the war period." Perhaps the most tangible contributions came in the pointing out of the necessity for a familiarity on the part of all bacteriologists with methods of pure culture study; that anaerobic organisms could be streaked on the surface of solid media, preferably blood agar, in Petri dishes; surface colonies obtained and studied with the subsequent selection and inoculation of differential media in the ordinary procedure of identification. Above all that a bacteriologic study of unknown material was not complete until the question of the presence of anaerobes had been satisfactorily disposed of. Nevertheless, it is a matter of common occurrence to find that the knowledge reawakened by the war studies has lapsed and today the average complete bacteriologic examination of suspected materials consists of the isolation and attempted identification of the aerobic flora.

The methods to be presented in the following pages are largely based on those used by Professor Novy (1893) in his studies, particularly with reference to the isolation of B. novyi. Naturally, since that time many important improvements in technic have been made and such aids have been incorporated in the general procedures as soon as their practical value was demonstrated.

MEDIA AND APPARATUS

It is essential in anaerobic procedures as well as in studies dealing with aerobes to be able to isolate the organisms in pure form and subsequently cultivate them serially upon suitable media, for only in this way is a knowledge of their function possible. A uniform set of cultural tests is of great advantage in distinguishing the various species and for this purpose the following media have been found to possess distinct value.

Litmus Milk.—To fresh skim milk is added a very concentrated aqueous solution of purified blue litmus sufficient to impart a decided blue color. After thorough mixing it is placed in test tubes to a depth of two or three inches and sterilized in the Arnold sterilizer for thirty minutes on each of three successive days. It can be sterilized in the autoclave at 110° C. for ten minutes.

Too much heat should be avoided, since it may cause an alteration of the milk. Milk is difficult to sterilize because of the presence of thermophilic organisms. Hence, it is advisable to place the tubes containing the milk at 37° C. for several days to make sure of its sterility. Litmus milk is used to ascertain whether an organism can coagulate casein, and whether it can decompose the lactose, thus giving rise to gas bubbles or to acid products. The medium when correctly prepared should have a lavender color, which turns red in the presence of acid and blue when bases predominate.

Coagulated Serum.—The usual horse or beef serum medium coagulated on the slant in test tubes is used to determine the liquefying power of the cultures.

Litmus Gelatin.—The ordinary 10 per cent nutrient gelatin containing purified litmus is used. Certain species liquefy gelatin and the litmus acts as an indicator of acid or alkali production, and in addition it may be reduced.

Blood Agar Plates.—The ordinary beef infusion agar is used as a base. The agar is melted, cooled to 50° C., and 20 per cent of defibrinated blood (horse, cow, human, or rabbit) added. The medium is then thoroughly mixed and poured into Petri dishes under aseptic conditions, and allowed to cool. A dry surface is essential to avoid a confluent growth. Well-isolated colonies are obtained in from twenty-four to forty-eight hours when a droplet of inoculum is placed on the surface of the medium, spread with a bent glass rod, and incubated under anaerobic conditions.

Cooked Meat.—Carefully trimmed heart muscle* (beef) is finely comminuted, added to 2 parts of distilled water, and stored in the ice box overnight. One per cent peptone and 0.5 per cent salt are added and the infusion is heated in the boiling water-bath for one hour. The water lost by evaporation is replaced and the reaction adjusted to P_H 7.4 with N/1 NaOH. The medium, tissue as well as extract, is placed in test tubes and sterilized by the fractional method for thirty minutes on each of five successive days. The tubes which contain some 2 inches of cardiac muscle overlaid with about half an inch of broth are incubated several days to insure sterility. Sterilization may be carried out in the autoclave if care is exercised to avoid the blowing out and spattering of the cotton plugs by the tissue. This is a very valuable medium for the cultivation and identification of anaerobes. The tissue supplies useful nutritive substances to the medium and it also acts as a reducing agent and thus removes any dissolved and toxic oxygen. Characteristic changes in reaction, color of the meat, and varying degrees of digestion also occur which aid in the differentiating of the members of this group.

Dextrose Agar, Dextrose Broth.—The usual beef infusion media containing 2 per cent dextrose is used. The former is very useful for plates, shakes, and stabs. The broth is used for making dilutions and on the addition of 5 per cent sterile blood is valuable for growing anaerobes direct from the animal body.

The above media, when used in test tubes, are placed just before inoculation in the boiling water-bath for five or ten minutes to drive out the dissolved air and are then cooled to 40° C.

*Certain workers prefer to use a medium containing brain tissue.

The pipette of the Pasteur School is to be preferred to the platinum loop for the inoculation and transferring of the cultures. The pipette is employed to transfer liquid cultures, pus, blood, transudates, etc., or surface growths from solid media. In the latter case a drop or two of sterile broth is mixed with the colony or growth, and a fine suspension made and drawn up into the pipette. It is a common experience that larger quantities of anaerobic material must be transferred to get a good growth of organisms than are necessary when working with aerobes.

The inoculum is always discharged into the bottom of the medium in the tubes. No overlaying seal to keep out the air is absolutely necessary, since reducing substances are present in the milk, gelatin, and meat media. Sterile yellow petrolatum to a depth of about one centimeter is in quite general use and forms a very excellent seal when placed on the top of these media. In addition to the exclusion of air, it prevents desiccation. The presence of the seal does not seriously interfere with the inoculation or withdrawal of medium, as the end of the pipette will readily penetrate the petrolatum. The dextrose agar tubes are usually thoroughly mixed after inoculation and used for shake cultures.

Culture Tubes.—The plain test tubes (15 by 150 mm.) of resistance glass are employed. They are thoroughly cleaned, plugged with cotton, and sterilized in the dry heat oven before the introduction of the medium.

Jars for Plate Cultivation.—The Novy jar,* with the special cock for vacuum work, is to be preferred. The following directions apply to this apparatus; vacuum desiccators and museum jars have been used and found satisfactory, but not as convenient as the specially designed jar.

The lower half of the jar should have an internal diameter sufficient to allow the easy introduction and withdrawal of the ordinary 100 mm. Petri dishes. Jars of this type are made in two sizes; one with an internal diameter of 13 cm. and an internal height of 12 cm.; the other has the same diameter with a height of 20 cm. The shallow jars will hold from six to eight Petri dishes and the deep jars will accommodate from twelve to fifteen plates. These jars are also used when large numbers of tube cultures are to be incubated at one time. The actual procedure is as follows: The surface of the medium, blood agar or glucose agar, is inoculated with a droplet of material from a pipette (poured dilution plates are frequently made). The inoculum is then spread, using a sterile bent glass rod. The plates are stacked in the lower portion of the jar and on top of the pile of plates is placed one of the halves of a Petri dish containing ten or fifteen thin slices of freshly cut raw potato, prepared with no attempt at sterility. The respiration of the potato, when the cover is placed on the jar, supplemented by that of aerobic organisms present will remove the traces of residual oxygen in the confined space and growth of the spores and vegetating forms will take place. There are objections to the use of excessive amounts of raw potato in the jars. Water is a respiratory product and the confined atmosphere, after a time, becomes supersaturated,

*Novy jars unless made according to the original specifications are unsatisfactory. They can be obtained from Greiner and Friedrichs, Stützerbach im Thüringen, Germany.

with the result that the condensation of moisture takes place on the agar surfaces thus favoring a confluent growth rather than the desired isolated colonies. In addition, there is ample proof that a definite tension of carbon dioxide favors the growth of aerobes and anaerobes alike, but excessive concentrations of this respiratory product serve as an inhibitory factor because of the acid nature of the substance in solution. The flanges of the jar are lightly greased with *lubriseal*, the cover put in place, a rubber band (16 by 130 mm.) is applied to the circumference, and six or eight small vises are then carefully and evenly tightened to the flanges. The main stopper should be wired in place. The jar can be made air-tight with very little care. The objection usually directed at the Novy jars is the difficulty of opening them after they have been in the hot room for several days. It is possible to loosen the top piece with an eccentric lever which is applied to the upper flange under moderate sustained pressure. The jars can be easily opened and breakage rarely occurs when warm water is applied to the flanges.

After the jar is closed and sealed, the glass cock is connected to a source of carefully washed hydrogen. This gas is best prepared in a Kipp generator, using dilute H_2SO_4 and granular zinc. The hydrogen so generated is passed through a series of four wash bottles containing alkaline lead acetate, 5 per cent solution of potassium permanganate, 5 per cent solution of silver nitrate, alkaline pyrogallate, and then into the culture jar. The gas must flow at a slow rate so that all impurities will be scrubbed out.

A vacuum line with a manometer and stopcock is joined to one end of the special stopcock on the jar. The purified gas is led through an auxiliary stopcock into the other end of the special stopcock on the jar. To fill the jar the gas supply is turned off and the vacuum line is opened; a vacuum of about 600 mm. is quickly drawn on the jar, the stopcock on the vacuum line is closed, and gas is led slowly into the jar through the open stopcock on the gas line until the negative pressure in the jar reaches zero. This latter stopcock is now closed and a vacuum again drawn in the jar with subsequent refilling with the washed gas. This procedure is repeated five times, at the conclusion of which only traces of free oxygen are present in the atmosphere of the jar. The jar is sealed by turning the stopper and then placed in the incubator. The small amount of oxygen still present will quickly disappear due to the respiration of the potato. After an incubation period of from two to four days, the jars are opened and the plates examined. Well isolated colonies are selected and transferred with a pipette to the several media.

The catalyst principle for the removal of oxygen from the container or jar was introduced during the war and it has found considerable favor with a number of workers. The active unit is ordinarily composed of asbestos impregnated with palladium or platinum and is suspended from the cover of the jar with suitable wire connections for attachment to the source of electricity which is used to activate the catalyst when the jar is closed. The inoculated plates or tubes are placed in the jar, the cover is attached, and the confined atmosphere is replaced with hydrogen. The electricity is turned on to heat the catalyst and the residual oxygen combines with the hydrogen under these conditions.

The heat is usually continued for at least half an hour and then the wires are disconnected and the jar is placed in the incubator.

Unsatisfactory results have been experienced by various investigators with the inert gases used to displace the air in the jars. Hydrogen, as obtained from the electrolysis of water, and nitrogen, a by-product of the liquid air industry, are available commercially under pressure in large drums. The ease of handling the gases in these containers and the necessity for the constant refilling of the Kipp generators when large volumes of hydrogen are used quite naturally encouraged many workers to secure these gases direct from the tanks and lead them without purification into the jars to displace the air. The unfavorable growths obtained when these tank gases have been used were unquestionably due to the presence of oxygen. It is the exception to find on analysis an oxygen content of less than 2 per cent in ordinary tank nitrogen. Samples from several tanks have had an oxygen concentration as great as 6 per cent and the ordinary tank hydrogen assays only a little better as far as oxygen content is concerned. Tank gases should be passed through an oxygen absorbent before they are led into the jars, or larger quantities of raw potato must be placed in the jars to take up the undesirable oxygen introduced. Traces of the lubricating oils used on the compression machines are also present in the tank gases and should be removed along with the oxygen.

GENERAL PROCEDURE

The exact procedure to be followed in the determination of the anaerobic flora of infected material depends quite naturally on the data desired and the substance under investigation. For example, when the presence or absence of one particular germ is routinely desired in a certain substance, such as B. welchii in fecal material, a procedure limited to the detection of this organism is usually followed. On the other hand, when the isolation and identification of the complete anaerobic flora are demanded, a technic must be employed which will take into consideration the nature of these organisms and the various interfering and inhibiting influences.

Infected material containing a pure culture of only one organism is the exception rather than the rule, and materials to be examined for anaerobes are usually rich in aerobes. Many difficulties attendant to the isolation of anaerobes from cultures containing aerobes as well as the separation of an anaerobe from a mixture of anaerobes and the various possible combinations must be given consideration. Over thirty pathogenic and forty saprophytic species of anaerobes have been described and almost every member of this group, singly or in combination with others, has been isolated from a wound infection at some time or other.

Consideration should be given to the following possibilities:

Sporulating and Nonsporulating Anaerobes.—Blood from human and animal sources and tissues from artificially infected animals usually come under this heading.

Sporulating Anaerobes With Nonsporulating Aerobes.—These are found in infected muscle removed at operation, pus from wounds, postmortem substances—

in brief, in material of human and animal origin. As a rule, such material is rather simple to handle. If the preliminary microscopic examination demonstrates the presence of spores, selective heating at 80° for twenty minutes is sufficient to remove such forms as the staphylococci, streptococci, B. proteus, B. pyocyaneus, and the coli forms.

Sporulating Anaerobes With Sporulating Aerobes.—Specimens of soil, water, milk, milk products, and the various pickled and preserved foods generally contain sporulating aerobes. Most of the aerobic spore formers grow very slowly under anaerobic conditions.

Nonsporulating Anaerobes With Sporulating and Nonsporulating Aerobes.—Naturally this combination is more difficult of separation. Heating is obviously out of the question. Primary enrichment (presented later) followed by surface cultivation with the picking and subculturing of a considerable number of the several varieties of colonies obtained ultimately yields pure strains. The procedure is time-consuming, tedious, and often disappointing.

A sample sufficiently large to inoculate several tubes of medium should be taken. Samples of food products if distinctly acid are neutralized before inoculating; the same for specimens of soil.

Before proceeding to the microscopic or cultural studies a general examination of the suspected material should be made. The origin of the specimen and its color and odor should be noted. A cover slip or hanging drop preparation is made. In the case of solid materials, such as necrotic tissue, the material is touched several times to a droplet of water on a glass slide, a cover slip is subsequently dropped onto the preparation, and it is ready for the microscopic examination. Semisolid substances, such as pus, feces, and sputum, are first emulsified with several drops of water, while clear fluids, such as urine, spinal fluid, etc., are centrifuged and then a portion of the precipitate submitted to direct examination. Care should be exercised especially in noting the form and grouping of the cells, the presence or absence of motility, the appearance of the protoplasm, and the presence of spores. While it is well known that abnormal forms frequently occur and that in the animal body departures from the typical morphology are frequently found, it is true that the information gained in this study may point the way later to forms that might be overlooked.

Methylene blue and Gram stains should be made of fixed films. The choice of Gram technic depends upon the nature of the material. It has been our experience that if one masters and routinely follows one procedure for this differential stain rather than changing the method to suit the material, the results are of greater value. Large gram-positive rods in pathologic materials are apt to be anaerobic bacilli. At best the results obtained from the preliminary direct and tinctorial examinations are merely suggestive.

Quite naturally the primary aim is to preserve all the anaerobes present in the sample taken, and for this a medium as little selective as possible should be used. The dextrose litmus gelatin medium was found for a long time to be very useful for this purpose, but it was ultimately replaced with the cooked meat medium. It has been pointed out that certain organisms, such as B. welchii, B. fallax, and B. aerofetidus tend to die out rather rapidly in a

dextrose-containing medium. The cooked meat medium is not only of importance because of its nutritive value and stimulating action on the growth of all germs but, in addition, the content of buffer substances makes it most suitable for the preservation of the microbic flora; even the less resistant forms will remain alive in this medium for years, so that one may return as often as desirable for subcultures.

Two tubes of the cooked meat medium are heated in the boiling water-bath for five minutes and then cooled to 40° C. A sample of the unknown is transferred to each of the tubes and the contents are then thoroughly mixed. The medium is then overlaid with sterile petrolatum. One tube is placed immediately at 37° C., the other tube is heated to 80° C. for twenty minutes and then placed at 37° C. The former tube gives an index of the anaerobic and facultative anaerobic flora and the heated tube will account for the sporulating forms.

Observations are made at frequent intervals during the incubation period for evidence of growth, such as gas formation, color, and possible digestion of the meat tissue. The richness of growth will depend upon the original inoculum.

At the end of twenty-four hours hanging drop preparations and stains are made from both tubes. If growth is absent, incubation is continued for at least two weeks before discarding the tubes.

If growth is present, glucose agar shake tubes are prepared. Tubes of glucose agar are melted and cooled to 45° C., a drop or two (depending on richness) of the culture is transferred to a tube of the melted agar and thoroughly mixed. From this tube two serial dilutions are made in the same medium and after thorough agitation the medium is permitted to solidify. Half-inch seals of sterile agar are superimposed on the medium and the tubes are incubated at 37° C.

Three blood agar plates are inoculated with droplets of the culture from each of the original tubes and quickly spread with a sterile bent glass rod, the amount of inoculum transferred again depending on the richness of the inoculum. The six plates are placed in a jar, the jar closed and at once subjected to anaerobic conditions, and then placed in the incubator.

Incubation of the two original inoculated tubes of meat medium is continued with frequent observations. It has been found that if a mixed growth of anaerobes occurs in a tube of meat medium the germs first to develop are B. welchii, vibrion septique, and B. fallax. This takes place within the first twenty-four hours. At the end of forty-eight hours' incubation the predominating organism will be B. sporogenes and a third phase of growth occurs at the end of ninety-six hours when B. tetani predominates. Thus, agar shakes and plate cultures made at these three intervals are most likely to give satisfactory isolations.

After forty-eight hours' incubation the plates are removed from the jars and the surface colonies examined microscopically with the low power lens. It is extremely important not to rely on the unaided eye in a consideration of colonies. One which appears to be well isolated macroscopically, may, on examination with the lens, be surrounded by many others or it may be seen to have de-

veloped in the midst of a surface film. After hanging drop examination and stained preparations have been made, well-isolated colonies of each variety present are transferred with a pipette to selective media, such as cooked meat, litmus gelatin, litmus milk, coagulated serum, and agar stabs. These subcultures are incubated and examined at regular intervals and the changes are recorded. It should be emphasized that changes in the media go on for a much longer period in the case of anaerobes; their full extent cannot be expected under ten or fifteen days. It is extremely important to control every anaerobic isolation for the presence of aerobes by inoculating an agar slant and incubating it under ordinary aerobic conditions.

The colonies in the agar shake tubes are examined with the hand lens and if found to be well separated, several of each type, if more than one variety is present, are selected, a slight scratch in the glass is made with a file directly over the colony and the edge of the scratch is touched with a hot glass rod thus causing the tube to crack, thereby exposing the medium which is placed in a sterile Petri dish. A sterile knife is used to slice the cylinder of medium in close proximity to the colony. Tubes of selective medium are inoculated from the colony and on incubation one rarely fails to secure a pure culture. It is probably true that this method gives the most consistent results in the hands of the beginner who is usually unappreciative of the strict requirements of the plate method.

The criterion for purity is the consistent behavior of a culture when submitted to growth on different media over a long period of time.

IDENTIFICATION

Identification may frequently be anticipated from the origin and nature of the specimen as well as from the data accumulated during the isolation. Short cuts are often resorted to, such as the direct inoculation of milk with a bit of the original material. The occurrence of a characteristic stormy fermentation in from twelve to twenty-four hours is diagnostic of B. welchii. It is advisable, however, to carry through the routine procedure. The characteristics available as a basis for identification can be grouped under the following categories: morphology, cultural, biochemical reaction, serologic reaction, the production of characteristic toxins, and pathogenicity. No one of the above aspects taken by itself furnishes sufficient data for a satisfactory identification. Thus, a combination of these various attributes has to be relied upon.

Information regarding the size, shape, and structure of the cells is usually obtained from an examination of the purified strain in the hanging drop as well as in stained specimens. Considerable care must be exercised in the judgment of motility. A direct drop quickly covered with a 20 mm. cover slip will furnish a satisfactory preparation for the determination of this character if examined at once. The special staining methods used for the demonstration of flagella may also be employed. The socalled "Giant Whips" are ordinarily seen in cultures of the motile anaerobes. The presence or absence of spores, and their position, are also noted in the direct drop and stained preparations.

The type of growth in broth should be recorded at frequent intervals. The examination of surface colonies has already been referred to. If more than one type of surface colony is observed, it usually indicates the presence of two or more species. Frequently, however, unlike colonies ultimately prove to belong to the same species. Atypical colony form has been frequently reported, but researches concerning the significance of these observations have until recently been held in abeyance. It is quite probable that investigations in this field will in the near future demonstrate that the phenomenon of microbic dissociation as found among the aerobes occurs in the anaerobic group and with like significance.

The determination of the capacity to decompose proteins and carbohydrates is quite readily detected. The proteolytic characters are judged by the extent to which coagulated serum and gelatin are liquefied. Gelatin tubes must be placed in the ice box following incubation of the culture in order to detect the extent of liquefaction. B. sporogenes and B. histolyticus are examples of the spore-forming proteolytic anaerobes which incite protein decomposition. The degradation products include indole, skatole, phenols, ammonia, hydrogen sulphide, etc., which in combination give a profound odor to these cultures. The proteolytic strains impart a black color to the cooked meat medium, and cause digestion of the tissue.

Saccharolytic power is recognized by a definite capacity for producing acid, or acid and gas in sugar containing media. The carbohydrate to be investigated is usually prepared in a 10 to 20 per cent aqueous solution and sterilized. It is then added with aspetic technic to tubes of sterile "sugar free" beef infusion broth. It is customary to employ the sugar in a concentration of 1 per cent. After heating and cooling the medium in the usual manner previous to inoculation, the germs are introduced and incubation is carried out until a rich growth is obtained. To detect acid production a few drops of the culture are removed to a test plate and an indicator, such as phenol red, is added. It has not been found satisfactory to incorporate the indicator in the culture medium. It must also be remembered that some anaerobes produce gas from sugar-free media.

Agglutinins, precipitins, and complement-fixing antibodies can be elicited with many species of anaerobes in the blood of experimental animals, particularly rabbits, by the intravenous injection of washed bacilli. The germs are cultured in dextrose broth for about forty-eight hours, collected by centrifugation, washed three times with sterile saline, suspended in saline, killed by heat, and then injected. Each animal receives from five to ten doses at intervals of from three to five days. The saline suspension of organisms may be used equally well for testing such serums. The most contradictory results are obtained as to antibody response. Ordinarily, however, satisfactory results are obtained with cultures of B. tetani and B. botulinus.

Toxin production is usually carried out by the inoculation and incubation of dextrose broth. The time of appearance of the toxin varies with the strain and the species. Thus, with B. welchii the most potent preparations are obtained after eighteen to twenty-four hours at 37° C. On the other hand, it

is necessary to incubate cultures of B. botulinus for at least ten days before obtaining satisfactory toxic material.

Antitoxins active against the respective toxins may be produced by inoculating animals with increasing doses of the corresponding toxin. The larger animals, such as goats and horses, are to be preferred for this purpose, as rabbits and guinea pigs are most difficult to immunize actively. It is customary therefore to obtain antitoxic serums from the commercial houses. These serums are kept on hand so as to be available for neutralization tests when injecting animals. A guinea pig is passively immunized by injection with antitoxic sera, or 1 c.c. of the culture in question is mixed with an equal volume of antiserum and left in contact for one hour at room temperature before intramuscular injection in guinea pigs.

For the determination of pathogenicity, a guinea pig is usually injected intramuscularly with 1 to 2 c.c. of a forty-eight-hour culture.

GENERAL CONSIDERATIONS

The above technic has been used over a long period of time and has been found to be simple, convenient, and satisfactory for the isolation and identification of the various pathogenic and nonpathogenic anaerobic organisms. The individual worker will quite naturally select procedures for his own particular requirements. He will find the methods capable of modification and adaptation in a variety of directions.

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TECHNIC FOR THE ISOLATION OF STREPTOCOCCI*

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WITHIN the past decade, streptococci have assumed increasing recognition as primary or secondary pathogens in a great variety of diseased conditions, and the problem of their prompt isolation and identification has become one of growing importance to the clinician. The purpose of this article is to discuss the bacteriologic procedures essential to the successful cultivation of these organisms. Many of the details described may seem elementary, but because of their importance it seems advisable to include them.

CLASSIFICATION

Since the days of Pasteur, considerable time and effort have been spent in attempting to classify the various forms of streptococci. Much of this work, while of interest to the bacteriologist, is of little practical importance. This applies especially to the defining of the fixed types of streptococci according to their power to ferment carbohydrates. Investigation has shown that there is little correlation between pathogenicity and fermentative activity. The classification of James Howard Brown is both practical and simple, and is based on the growth of deep colonies of streptococci on blood agar plates. His three main groups with their approximate synonyms are as follows:

1. Alpha type**: colonies surrounded by green zones with partial hemolysis of the blood corpuscles—Streptococcus viridans.

2. Beta type: colonies surrounded by definite, clear, colorless zones of hemolysis—Streptococcus hemolyticus.

3. Gamma type: colonies producing no change in the medium—indifferent streptococci.

CULTURE MEDIUMS

Many special and complicated mediums including the use of sugars and various animal tissues have been advocated for the growth of streptococci. For practical purposes, beef-heart infusion broth and beef-heart infusion agar are the most satisfactory.

Preparation of Broth.—Fresh beef heart, free from fat, is passed through a meat grinder and weighed. To every 500 grams of meat one liter of tap water is added. It is then boiled, with frequent stirring, for fifteen minutes and is placed in the ice box over night. The following morning it is heated to 20° C., and filtered through a flannel bag. One per cent bacto-peptone and 0.5 per cent sodium chloride are added. The mixture is then heated for a few minutes over a low flame, with constant stirring, until the peptone is dissolved. The hydrogen ion concentration is adjusted to a P_H of 7.9 with 2N sodium hydroxide.† The solution is placed in the Arnold sterilizer at 100° C. for one hour and then passed through a filter paper. The P_H is again taken and is usually found to have fallen to approximately

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**Brown also describes an alpha prime type of streptococcus which is intermediate between the alpha and beta types in character.

†The authors have found the "Hellige" comparator satisfactory for P_H determinations.

7.6. If it has fallen much below this point, a further adjustment is necessary, followed by another hour in the Arnold sterilizer. The medium is placed in tubes and bottles and sterilized in the Arnold sterilizer for twenty minutes at 100° C., on three successive days. The broth is then incubated for twenty-four hours to test for sterility. The final P_H should be between 7.4 and 7.6. For purposes other than blood cultures, the medium is enriched with 1 per cent defibrinated blood.

Preparation of Agar.—The agar medium is prepared in the same way as the beef-heart infusion broth, with the exception that 1.5 per cent of bacto-agar is added with the peptone and sodium chloride. One per cent defibrinated blood may also be added, for enrichment, prior to use.

Huntoon's "hormone" broth and agar and Bailey's modification of them provide mediums rich in vitamines and are particularly suitable for the growth of streptococci. However, they have the disadvantage of being slightly cloudy and are a little more difficult to prepare.

There are a few general principles that are important in the successful cultivation of streptococci. They grow best in a slightly alkaline medium (P_H 7.4 to 7.6). The optimum temperature for growth is about 37° C. Growth is not always obtained in forty-eight hours but may occur after ten days or even longer. The tendency to discard cultures too early accounts for many negative results. Practically all streptococci grow well aerobically, but a number of them have been found which grow more luxuriantly under anaerobic or partially anaerobic conditions. The more general use of supplementary anaerobic technic is recommended.

MICROSCOPIC EXAMINATION

The microscopic study of stained smears is, of course, an essential part of every bacteriologic examination. For this, the Gram method is the most useful for general purposes. The smear should be thin, even, and fixed by passage several times through a flame. The actual technic of staining is adequately described in standard textbooks. In inexperienced hands the results are apt to be misleading, due to insufficient decolorization, to improperly prepared, or old stains, or to uneven distribution of the stain on the fixed film. Control smears should always be made with gram-negative and gram-positive organisms and placed beside the film to be examined. Stock slides prepared with smears from actively growing cultures of Staphylococcus aureus and Bacillus coli are frequently used for this purpose. These tend, however, to deteriorate and lose their staining characteristics within a few weeks. A satisfactory control may readily be obtained from the debris between the teeth. This material almost always contains gram-negative and gram-positive organisms. No control method is entirely free from error, as substances may be present in the original specimen that interfere with the staining process. The methylene blue stain is valuable when the study of the morphology of organisms is desired.

INOCULATING MEDIA

The best results with the use of liquid media are obtained when the amount of inoculating material compared with the amount of medium does not exceed the ratio of 1 to 10. In culturing materials, such as pus and serous fluids, it is advisable to seed several tubes of blood broth, using amounts of the

inoculum varying from a few drops to 1 c.c. Cultures containing the smaller amounts will occasionally yield positive results while those more heavily inoculated remain sterile, and vice versa. Liquid media are unsuitable for contaminated specimens or those suspected of containing more than one type of organism.

The technic for streaking blood agar plates depends on the nature of the specimen. Throat swabs usually contain a variety of organisms, and for successful culturing a thin streak is necessary. To accomplish this the swab is permitted to touch but a small area of the medium and is then discarded. A fresh swab is passed once through the inoculated area and streaked back and forth across the plate or in the form of a fan. In culturing material in which only streptococci are present the streaking may be done with a platinum loop. After passing through the inoculated area, the loop is pushed through the blood agar and a horizontal cut made. It is then withdrawn and streaked over the surface. This method permits the study of deep and surface colonies on the same plate. When the specimen is suspected of containing few or no microorganisms, the plate is streaked in the form of a fan, the swab or platinum loop being passed through the inoculated area at each stroke.

THROAT

The usual method of taking cultures from the throat is carried out with the aid of a sterile cotton swab at one end of a wooden or wire applicator. When localized inflammation exists, care should be taken to swab only the inflamed area. It is unnecessary to squeeze exudate from tonsillar crypts or to puncture them with a hollow needle, as it has been found that such procedures give substantially the same results as ordinary swabbing. The infected material is streaked on plates as previously outlined.

It should be remembered that in healthy persons green-producing streptococci may be recovered from the throat in 90 per cent of cases and hemolytic streptococci in from 10 to 20 per cent. In acute infections of the throat hemolytic streptococci are almost always present.

TONSILS

Excised tonsils which are to be studied bacteriologically should be sent directly to the laboratory without being opened by the surgeon. Each tonsil should be cultured separately. In our experience, the following technic has yielded satisfactory results. The tonsil is immersed in 95 per cent alcohol for one minute, removed with sterile forceps, shaken once to remove excess of alcohol, and placed in a sterile Petri dish. It is then cut open with sterile instruments and a piece of the central portion removed and streaked on blood agar. Another piece is incubated in blood broth. If the technic is carried out carefully, the mouth organisms are avoided, and pure cultures of streptococci are frequently obtained.

TEETH

Dental infections are usually streptococcal in origin. Before extraction, the gums should be painted with iodine and 95 per cent alcohol. In removing

the tooth, care should be taken to avoid contamination from other mouth structures. The apex is immediately rubbed over the surface of a blood agar plate. The tooth is then dipped in 95 per cent alcohol for one minute and cracked open in a sterile towel with bone forceps. The interior of the root canal, granuloma and abscess if present, are cultured on blood agar plates and in blood broth tubes. Pieces of tooth may also be cultured in blood broth. By this method streptococci are almost always found in diseased teeth and generally in pure culture.

SPUTUM

To examine sputum for streptococci, thin smears are prepared, stained with Gram's stain and methylene blue and examined under the microscope. Mucoid material may be streaked directly on blood agar plates. Mucopurulent masses, after washing in sterile water, should be emulsified in nutrient broth or saline before culturing on blood agar.

PUS

Pus may be collected with a platinum loop, sterile swab, bacteriologic pipette, or a syringe, and a needle. Smears are stained with methylene blue or Gram's stain. Cultures are made on blood agar plates and, if the material is not contaminated, in blood broth also. The method, previously described, of inoculating several tubes with varying amounts of pus is particularly useful.

SINUSES

Sinus material submitted to the laboratory for culture is either in the form of sinus washings or of pus which the surgeon has collected on a swab after puncturing the sinus. It is assumed that the operator has attempted to sterilize the nose as thoroughly as possible before puncturing the sinus. Cultures are made on thinly streaked blood agar plates and in blood agar pour plates.

JOINT, PLEURAL, PERICARDIAL, AND PERITONEAL FLUIDS

The process of obtaining joint fluid by aspiration subjects the patient to possible danger through the introduction of pathogenic microorganisms. To insure a sterile technic, extraordinary precautions must be observed. Any necessary palpation for the location of landmarks should be carried out before sterilizing the skin. When the aspiration is performed at the bedside, the use of sterile gloves is not advised, as they tend to give the operator a false sense of security. A sharp needle of 18 or 20 gauge is selected. Sterilization of syringe and needle in a dry sterilizer or autoclave is preferable to boiling. The skin is painted with two coats of iodine and one of alcohol, the iodine being permitted to dry each time before the application of the next coat. Knee aspirations are usually carried out on the medial aspect of the joint immediately below the patella. Other joints are usually aspirated at the point of greatest fluctuation. When joints contain a very small amount of fluid, it is permissible to wash them out with a little sterile physiologic saline which enables the operator to obtain a specimen otherwise impossible.

With sensitive or neurotic patients it may be advisable to use a local anesthetic. A hypodermic syringe and 2 per cent novocaine from an ampule are used for this purpose. After a small bleb is made in the skin, the novocaine is slowly injected while the needle is being inserted to the joint capsule. Five to ten minutes should then be permitted to elapse in order to give the anesthetic time to take effect before proceeding with the resterilization of the skin and aspiration. Novocaine injected in this way does not interfere with the culture.

Smears are made and stained in the usual manner with methylene blue and Gram's stain. In addition, it is sometimes advisable to use Wright's, or some supravital, technic, for study of the cell content. Cultures are made in blood agar and blood broth as previously described.

The bacteriologic examinations of pleural, pericardial, and peritoneal fluids are carried out in the same way as for joints.

URINE

In culturing the urine, it is necessary to use a catheterized specimen, and considerable attention should be paid to the sterilization of the urethral orifice. Cultures are made on blood agar plates and in blood broth tubes. It is always advisable to inoculate several tubes of blood broth with varying amounts of urine.

GENITAL TRACT

Bacteriologic examination of the female genital tract, other than the uterus, is usually made by studying smears stained with Gram's stain and methylene blue, and from cultures on blood agar plates. Examinations of the uterine cavity are complicated by the possible danger to the patient through the introduction of pathogenic microorganisms. The technic of Harris and Brown is the method of choice for cases of suspected puerperal sepsis. According to this procedure, the external genitalia are painted with 2 per cent alcohol-acetone solution of mercurochrome. The cervix is then exposed with a sterile bivalve speculum and wiped dry with sterile gauze sponges. A glass lochial tube of the type described by Little is inserted in the uterine cavity and carried to the fundus. When the tube is filled, it is removed and immediately taken to the laboratory. The tube is broken with the aid of a file and the middle of the column of lochia is examined by smears and culture. Blood agar plates and slants sealed under reduced oxygen tension, human serum bouillon and cooked meat medium sealed with vaseline are used. The last medium gives the best results. It must be borne in mind that streptococci are occasionally found in the vaginal secretions of normal females.

STOOLS

The culturing of stools is complicated by the presence of various kinds of bacteria, in large numbers. Many methods for growing streptococci have been advocated by investigators. The principles generally employed are either the dilution of the stool or the use of various reagents to inhibit the growth of unimportant organisms. Probably the most common technic calls for the dilu-

tion of the stool in physiologic saline before culturing on blood agar pour plates. In our experience, the following method has been found to be most satisfactory when only the streptococcal content of the stool is desired: A representative specimen of stool the size of a pea is emulsified in a test tube containing 10 c.c. of a 1 per cent solution of sodium carbonate and allowed to incubate at room temperature for twenty-four hours. Subcultures are then made on blood agar pour plates, using varying amounts of the inoculum for each plate. The cultures are then incubated for twenty-four to forty-eight hours at 37° C. This method eliminates all the bacteria usually found in stools except the streptococci.

It must be remembered that 90 per cent or more of stool specimens from normal persons contain green-producing streptococci, while 10 to 15 per cent of them contain hemolytic streptococci.

The streptococci found in the stools fall roughly into two groups: the so-called true streptococci and the enterococci. The latter may be distinguished by the following characteristics which are not found in the first group.

1. They ferment mannitol (Andrewes and Horder).
2. They grow well in bile-glucose-peptone broth (Weissenbach).
3. They are heat resistant (Dible).
4. They are able to split esculin (Harrison and van der Leck).

SKIN

Two methods are particularly efficient for the recovery of streptococci from the skin of erysipelas patients: (1) Fehleisen, after sterilizing the surface of the periphery of the lesion, excises a portion of the skin and cultures it in blood broth. By this method, a hemolytic streptococcus is readily recovered. (2) Birkhaug has successfully eliminated the necessity of an operative procedure by injecting 0.5 c.c. of sterile saline into the skin at the edge of the lesion; later, aspirating and culturing the fluid.

In culturing skin conditions where contaminations are present the Sabouraud bouillon pipette method and Haxthausen's crystal violet method are satisfactory.

Sabouraud cultures the serum exudate, or an emulsion of scales from the lesion, using ascitic fluid with bouillon in Pasteur pipettes. This method permits aerobic conditions in the wider part of the pipette and semi-aerobic conditions in the capillary stem. The latter is a more suitable environment for streptococcal growth, while it is unfavorable for the multiplication of staphylococci. After a period of twenty-four hours' incubation, the fluid in the stem of the pipette is examined and subcultured. In this way a pure culture of streptococci may be obtained from a mixed infection.

Haxthausen's technic allows for a quantitative estimate of the number of streptococci present, while at the same time it eliminates contaminating organisms. The medium he uses is a 10 per cent blood agar in which crystal violet in a concentration of 1:100,000 is added. This investigator states that in nine out of ten cases any streptococcus will grow in this medium, while staphylococci are entirely, or almost entirely, inhibited both when the culture is made from fluid material and when scales are sprinkled over the agar. A liquid bouillon medium may also be used with the same concentration of the dye.

TISSUES

The usual methods of staining bacteria in tissue sections are unsatisfactory in that frequently gram-negative organisms are not decolorized and that the bacteria are not clearly differentiated from tissue elements. Brown and Brenn have recently described a method that largely eliminates these difficulties, decolorization being accomplished with a solution of picric acid and acetone. Readers are referred to their original article for details.

Cultures are made by placing pieces of the tissue in blood broth and macerating them with a glass rod. Tough material may be ground in a mortar with sterile sand, preferably moistened with a small amount of bouillon, and then cultured in blood broth and blood agar. The grinding process, even under suitable conditions, exposes the tissue to possible contaminations and should be used infrequently.

MILK

In epidemics of septic sore throat the clinical laboratory is sometimes called upon to examine samples of milk. Blood agar pour plates are generally used for this purpose. Hardenbergh's technic is satisfactory. Samples from individual cows are generally cultured in dilutions of 1:100; composite samples, in dilutions of 1:20. For each plate, 1 c.c. of the diluted milk is used and the culture is incubated from eighteen to forty-eight hours. Organisms suspected of being Streptococcus epidemicus are tested with a medium containing sodium hippurate. Streptococcus epidemicus does not split nor hydrolyze the sodium hippurate, while the other streptococci do. In addition, the Streptococcus epidemicus has a capsule.

The estimation of the final hydrogen ion concentration of the medium is valuable in differentiating the human and bovine types of Streptococcus hemolyticus. If grown in 1 per cent dextrose broth the streptococcus of human origin will give a final P_H of 5 to 5.3 and that of bovine origin, 4.3 to 4.5 (Avery and Cullen).

BLOOD AND SPINAL FLUID

Blood and spinal fluid cultures are described in a separate article.

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PREPARATION OF VACCINES*

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SINCE the introduction of bacterial vaccines as therapeutic agents, their use has undergone the vicissitudes which not infrequently have attended the introduction of various other preparations in medicine. Frequently exploited by the commercial laboratory, hailed by the therapeutic faddist as a "cure-all," used blindly in the treatment of almost every conceivable ailment,[1] and often as a last resort in some obscure condition where other therapeutic means had failed, reaction naturally followed, condemnation often replaced praise, and their general usage diminished. Nevertheless, excellent therapeutic results were occasionally observed in the treatment of certain conditions which encouraged serious study of the problem by some investigators. The continued investigations of scientifically trained bacteriologists and immunologists in conjunction with discriminating clinicians have advanced the preparation and application of bacterial vaccines to a rational basis, and established their place in therapeutics among other well recognized biologic agents. Recent discoveries in the fields of bacteriology and immunology have shed light upon some of the past failures, and have tended to clear the problem. As knowledge has been gained concerning bacterial variations, and their relationship to antigenic properties, advances have been made in the preparation of more efficient products. Methods of cul-

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ture have improved, and the selection of the proper type organisms to enter the vaccine has become possible.

Experimental evidence indicates that the antigenic properties of bacteria are dependent upon certain chemical components of the cell, even associated with certain differentiated portions of the cell, the chemical nature of certain of these cellular products is known,[2] and the problem of specificity has been elucidated in certain instances.

Obviously, the desired objective in the preparation of an autogenous vaccine is to culture the organism under conditions which produce the least alteration in the finer chemical structure of the harmful parasite as it existed in the host; arresting or destroying its vitality with the least alteration of that chemical structure, and retaining the intrinsic composition of the antigenic components of the cell in that state in the prepared vaccine.

In the following article on the properties of autogenous vaccines it will be noted that, insofar as practical, the above mentioned principles have been applied. The methods employed have been the outgrowth of years of experience in this field, and have given satisfactory results. As knowledge advances, improvement in methods must follow; procedures now practiced may be found inadequate when viewed in the light of future investigations. Emphasis must be given to the importance of the strict supervision of the entire procedure by a well trained bacteriologist and immunologist. The average laboratory technician does not possess the training, nor the discrimination essential for the best results.

In recent years the use of autogenous vaccines as a means of treatment has been extended to include certain allergic conditions, possibly due to focal infections. Therefore in outlining a method of procedure, it is necessary to consider in detail foci of the respiratory, the gastrointestinal, and the genitourinary systems. A complete bacteriologic survey of the patient should be undertaken in most instances to secure the offending organisms.

Collection of Specimens.—Materials are collected from the nasal passage and accessory sinuses, either by aspiration under aseptic precautions, or by means of a sterile cotton swab passed along the floor of the canal after the patient has vigorously blown the nose on sterile wipes. A sterile nasal speculum should be used to dilate the nostrils, when swab specimens are taken. Secretions, etc., of the nasal pharyngeal area are collected by means of the West tube. In taking specimens for culture from the faucial tonsils, pains must be taken to express pus from possible pockets, and deeply penetrate the crypts to secure caseous materials, etc. The ordinary cotton swab (sterile) usually answers all purposes. Occasionally the lingual tonsils are involved; therefore they should be examined in the routine procedure. Early morning specimens of sputum, or that following paroxysmal coughing attacks, should be collected in a sterile jar, and kept on ice until cultured.

Roentgenograms of the teeth should be made, and when evidence exists of apical infections, these teeth may be extracted under aseptic conditions and cultures made. If possible, material should be taken from the socket and cultured on suitable media immediately at the time of extraction. Cultures should be made from gum lesions where pyorrhea is evident.

In some instances gall bladder infections are suspected. Bile specimens may be secured for culture by passing the duodenal tube and allowing it to remain until sufficient fluid is collected for cultural purposes. Aseptic precautions must be taken throughout to exclude, insofar as possible, extraneous contamination.

Fecal material for culture may be obtained by means of high colonic irrigation with sterilized water, or from the last movements following the administration of castor oil, or one of the saline cathartics.

Urine specimens may be secured by catheterization, or following normal passage, after having cleansed the external orifice with soap and water, and then rinsing thoroughly with sterile saline solution.

Not infrequently the vagina and the cervix are sites of infection, and should be routinely cultured in those suffering from chronic arthritis. The patient is placed in the proper position for examination, a sterile vaginal speculum is inserted, and by means of long cotton swabs, two sets of cultures are made, one from material taken from the vaginal fornix; the other from the os uteri.

In the male, the prostate may be involved. In such conditions, the external parts are thoroughly cleansed with soap and sterile water, then the gland is massaged, and the fluid collected on sterile swabs as it passes; then cultured at once, if possible.

Other possible foci of infection may be found and cultured. But those just mentioned constitute the group which must be considered in a general bacteriologic survey of the patient suffering in particular from an allergic symptom complex.

Culturing of Materials.—Before culturing materials from any source, if a sufficient amount of the specimen permits, a preparation stained by the Gram method is studied microscopically, and the various types of organisms present are noted, as well as their quantitative relationship to each other. As a routine procedure, materials from the respiratory tract are cultured directly in dextrose broth, on blood agar, and on hormone agar slant. Human blood is preferable in the preparation of the agar slant, and in the Avery medium. When possible, sputum is washed through three changes of sterile physiologic salt solution, then the washed masses are placed in the culture media. Also the washed sputum, pus, feces, etc., are emulsified in broth; then immediately plated with blood agar by the poured plate dilution method. Feces are cultured on plain and dextrose broth, and in 1 per cent sterile sodium carbonate solution; this solution in amounts of about 10 c.c. is placed in the ordinary test tube and sterilized in the usual way. Each tube of medium receives a quantity of material equivalent in size to a navy bean. Vaginal and cervical materials, and prostatic fluid, are cultured directly at the time of taking in dextrose brain broth (Rosenow). The medium is first placed in a cup of water, boiled for ten minutes, then cooled to 37° C. or less, before the material is added for culture.

After eighteen to twenty-four hours' incubation at 36 to 37° C., the growth in the various culture tubes is studied microscopically from prepared films stained by the Gram method, and the morphologic types are noted in each instance. Then the cultures are plated by the poured plate, dilution method with blood agar, for isolation of the species present. After proper incubation of the plate cultures, the colonies are studied, their general characteristics noted.

including their hemolyzing properties, "rough" or "smooth" appearance, etc., and then fishings are made to suitable media. Those resembling the streptococcus and the M. catarrhalis type colonies are cultured on dextrose broth; the staphylococcus type on plain agar slants, and the coliform colonies on both dextrose lactose agar (Russell) and saccharose mannitol agar (Kendall) for a preliminary differential test. Materials such as sputum, pus, etc., which were emulsified and directly plated, may yield organisms in pure culture by the second generation to be converted into a vaccine, while those first cultured on broth or agar media before plating usually undergo three or more generations before they can be prepared as vaccines.

On a priori grounds, it would appear important to immediately convert the organisms isolated from the patient into a vaccine for his treatment, on the supposition that the relation of organism to the host was that of a highly parasitic character, which would tend to be lost if the organism was grown a number of generations upon artificial medium, a saprophytic existence. It would appear that much of the finer specificity might be lost as the organism readjusted itself to a changed environment. For some years this principle in the preparation of autogenous vaccines has been practiced,[3] and probably with beneficial results. In limiting the number of generations of the organism as derived from the patient, the tendency to variation or mutation would be much lessened, a thing of theoretical importance, and probably of practical significance. Also in making fishings of colonies from plates, only the smooth type is selected, on the basis of their specific antigenic properties. Again, in some instances the question of oxygen requirement is important, and requires special handling of the organism.

Cultures for Vaccines.—The antigenic substances of bacteria are assumed to be associated with the nucleoprotein of the cell and the capsular and the flagellar structures when they are present. In culturing bacteria for vaccines, a selection of media which favor the development of the organism to its fullest extent is desirable. However, certain cultural media ordinarily used for such purposes might introduce substances of a harmful nature, and therefore should not be used. As a routine, the staphylococcus and the coliform groups are grown upon plain agar slants, although the latter group may be grown upon the double sugar media of Russell and of Kendall; the streptococcus, the M. catarrhalis and the diphtheroid groups are grown upon dextrose broth flasks containing approximately 75 c.c. of fluid. B. influenzae is grown upon 5 per cent human blood agar.

As mentioned above, the first or second generation of the organism should be used for seeding purposes, and as early as possible in its development. Incubation at 36° C. is found to produce excellent growths usually in eighteen to twenty-four hours in properly prepared media whose reactions are correctly adjusted. Often it is advantageous to place the freshly seeded media in a water-bath at 36 to 37° C. to hasten the growth. Young, vigorously growing cultures, eighteen to twenty hours old, should be used for the preparation of the vaccine, thus holding to a minimum autolytic disintegration, the deleterious effects of acid production, etc. The use of old cultures for seeding purposes, or for the preparation of vaccines, should not be permitted.

Preparation of the Bacterial Vaccine.—A modification[4] of the volumetric method of Hopkins[5] has been used in our laboratory for a number of years with highly satisfactory results. This method possesses the advantage of simplicity of technic, rapidity of results, relative uniformity of concentration, etc., which commends its use from the practical standpoint. The only special piece of apparatus required is the Hopkins centrifuge tube,[5] which may be procured from any of the leading scientific supply houses. However, it is necessary that these tubes meet the specifications laid down in the original article by Hopkins. The other apparatus used may be found or made in any well-equipped laboratory. No attempt will be made to enter into details in this article, as they have been published elsewhere,[6] but only a general outline of the procedure will be given. At the outset it must be stated that only sterile glassware, solutions, etc., may be used throughout, and strictly aseptic precautions exercised in each step. If the organisms are grown on agar slants, they should be emulsified in a small portion of physiologic salt solution. The bacterial suspension is removed by means of a Pasteur pipette, then strained through cotton in a suitable filter funnel, and collected in a Hopkins centrifuge tube. The filter funnels are prepared by drawing out test tubes, or using a Hopkins tube whose tip has broken off, and placing a small tuft of absorbent cotton in the broken end. Both the filter funnel and the Hopkins tubes are placed in larger test tubes, which are sealed with cotton, and sterilized by dry heat before using. If the organisms have been grown in broth flasks, the sediment is removed directly by means of a sterile pipette, and collected in the Hopkins tube. The tubes are sealed with gauze-wrapped cotton plugs, which are securely fastened in position; then placed in the centrifuge. The organisms are thoroughly packed in the collecting portion of the centrifuge tube after one-half hour in a machine carrying a head of 18 cm. diameter, driven at 2800 to 3000 revolutions per minute. After sedimentation is completed, the supernatant fluid and any excess of bacterial sediment are removed by means of a Pasteur pipette and rejected. Sufficient physiologic salt solution is added to yield a 1 per cent suspension of the bacterial residue, and a homogeneous suspension is made by thoroughly mixing with the pipette. The bacterial suspension is transferred to a sterile test tube containing a few small beads, which is thoroughly heated in a Bunsen flame from the mouth downward about one-half its length; then is sealed with a sterile cotton plug.

Killing of the Organisms.—Much has been written concerning the best methods of treating the organism before its introduction into the body of the individual to be immunized, whether the organisms should be simply attenuated, or should be killed.[7] Owing to the attending risks of infecting the recipient, attenuated cultures have fallen into disuse. At the present time, practically all investigators are agreed upon using organisms whose vegetative powers have been destroyed, even if the living organism theoretically possesses greater antigenic possibilities. As to the best method of arresting or destroying the vegetative powers of an organism and still retaining its highest antigenic value, much disagreement exists among different workers. Whether this should be induced by physical means, such as the production of autolysates[8] or by the use of heat, or,

on the other hand, by chemical means,[9] such as by germicides, still remains to be decided. The chemical group embraces a rather large number of substances, each appearing to have its special advocates.[10] In particular, many hold that chemical means of killing organisms is superior to the use of heat,[11] but the published studies are not fully conclusive, since the claims are frequently based upon animal experimentation and studies of certain immune bodies, or serologic reactions, which have a questionable relationship to the true immunologic processes occuring in the patient undergoing protective, or therapeutic immunization. The whole subject requires restudy, taking in consideration certain biologic reactive processes more closely associated with pathologic conditions, such studies are now under way. Therefore, until more conclusive evidence than that which has been presented up to this time is forthcoming, the heat-killing method will be retained in preparing the vaccine. But, as a basic condition, only the minimal amount of heat is used which just kills the organism after one hour exposure. In general, 58° C. suffices for the true streptococcus group, and some of the gram-negative cocci, but for the coliform group and the staphylococcus group, a higher temperature is necessary, and even a temperature reaching 64 to 65° C. may be required to insure the killing of the enterococcus (M. ovalis). The sterilized test tube containing the physiologic salt solution suspension of the organism is submerged to a depth of about one inch from the mouth in a water-bath, then heated for one hour at the required temperature. A separate vaccine is made from each of the organisms isolated from the various foci cultured in the patient.

Control of Vaccine.—After heating the suspension of the organisms as stated, the tube is removed from the bath and shaken thoroughly to ensure a uniform mixture, then two or three drops are planted into each of two culture tubes, one containing about 20 c.c. dextrose broth, the other an agar slant. These tubes are incubated at 36 to 37° C. for seventy-two hours to determine the sterility of the vaccine. After making cultures for the sterility tests, the remainder of the vaccine is transferred to a sterile, 15 c.c. amber glass bottle, containing a few small glass beads, and sufficient 10 per cent emulsion of tricresol in distilled water is added to give a final dilution of 0.25 per cent. The bottle is sealed with a sterile rubber stopper, properly labeled, then well shaken, and stored in the refrigerator. When the sterility of the preparation has been proved, the vaccine is ready for use. The vaccine in the storage bottle is known as the "stock vaccine," and contains practically 1 per cent of the killed bacterial substance.

Standardization of Vaccine.—Hopkins estimated the average number of organisms present in 1.0 c.c. of 1 per cent suspensions of various bacterial species prepared by his technic, and established those numbers as "standards." Dilutions of the "stock vaccines" were made for therapeutic purposes, and the bacterial concentrations were expressed in numerical values.

In a study of the application of autogenous vaccines to the diagnosis and treatment of asthma,[12] it was found that the numerical method of standardization as employed by Hopkins was not as practical as could be desired. Therefore a modification[4] was introduced which proved more satisfactory. The standard adopted was based upon the *volume* content of moist bacterial substance col-

lected in the Hopkins tube under standardized procedure. The *vaccine unit** may be defined as consisting of that volume of bacterial substance (killed) represented by 0.01 c.c. of a 1 per cent suspension, prepared under certain required conditions. This quantity (one unit) of vaccine has been found the most favorable amount to produce a positive skin reaction[13] in the hypersensitive individual when given intradermally; the absence of a reaction eliminates a patient as nonsensitive to the organism, insofar as the direct skin test indicates. The particular advantages of the volumetric vaccine unit are greater uniformity in dosage, constant valuation of vaccines prepared from the same species, and comparable valuations of vaccines prepared from different bacterial species.

Testing Vaccines.—Vaccines for the intradermal test of the patient to determine his reaction to the various organisms (killed) isolated from material taken in the bacteriologic survey, are dispensed from the 1 per cent sterile stock preparation. They are supplied in 1 c.c. ampoules, either as the original 1 per cent suspension, or a dilution[14] of one part of stock vaccine in five parts (1:5) of the standard diluent, consisting of sterile physiologic salt solution containing 0.25 per cent tricresol. Usually 0.5 c.c. of either suspension is placed in the sterile ampoule, which is sealed with sterile rubber stopper, then properly labeled, and kept in the ice box until used. The dose used in the skin test consists of 0.01 c.c. of the 1 per cent concentrate, or 0.05 c.c. of the diluted stock vaccine, the larger volume permits more accurate measurement.

Therapeutic Vaccines.—The preparation of the treatment vaccine is dependent upon the results of the skin reaction following the intradermal injection of the test vaccines. One or more bacterial species may be indicated as etiologic factors in the patient's condition, so the respective vaccines are selected accordingly for therapeutic use. They may be dispensed separately, or may be combined in different proportions as desired. Usually the concentration of the autogenous vaccine, primarily supplied, contains 10 units of the bacterial substance per cubic centimeter, but it may become necessary to alter this concentration after the first injections, depending upon the patient's general reaction to the treatment. The dilutions of the treatment vaccines are made from the "stock vaccines" by accurately measuring out the required amount of the uniform suspensions after thoroughly shaking, with a sterile 1 c.c. pipette marked with 0.01 c.c. graduations. The given amounts of the respective vaccines are placed in a sterile 10 c.c. vial, containing a few small glass beads, then that amount of standard diluent (see above) is added to bring to required volume, sealed with special sterile rubber stopper, then properly labeled. The vaccine is kept constantly in the ice box except when in actual use.

In presenting this discussion on the preparation of vaccines it is with the knowledge that certain procedures may be subject to just criticism, as much difference of opinion exists among different investigators as to the relative merits

*Occasionally certain individuals are found to be extremely hypersensitive to certain bacterial vaccines, and grave reactions follow the initial dose. In such cases, greatly reduced doses must be administered to them during the course of their treatment. As more or less difficulty may be experienced in dealing with fractional unit dosage, it has been suggested by Dr. William S. Thomas that a *milliunit* of measurement might be recognized, and thereby the dosage be expressed in whole numbers in that particular group of cases. By the *milliunit*, it is understood that the original, whole unit as defined above, is simply subdivided into one thousand parts, and each of these parts designated as a *milliunit*. The doses of vaccine administered to the patient during the course of treatment would be recorded on his chart in terms of milliunits rather than in fractions of the basic unit.—Personal communication.

of one or another method of arriving at a given result. Ultimate results have appeared to justify the methods outlined in the foregoing presentation. Revision may become necessary if further investigations definitely decide certain doubtful questions, and show their superiority to procedures now in vogue.

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VACCINES IN CLINICAL MEDICINE

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AT FIRST thought it would seem that a well rounded paper on the subject of vaccines would be both timely and easy to present. The difficulties and uncertainties of vaccines and vaccine therapy and the wide divergence of experimental and clinical opinion accounts for an imbalance in the position of the whole subject at the moment. We may rule out of this discussion the active immunity of vaccines produced in the prevention of such diseases as typhoid fever, since this is an established accomplishment, and we are no longer living in the age of Chicamauga Park as far as typhoid fever is concerned.

You either believe or you do not believe in vaccines. This attitude may be well grounded from actual first hand experience, it may be a belief fostered in ignorance, or it may be the result of a teaching which you follow. There is too much accomplished by the intelligent use of vaccines[1] to entirely disregard them, as is the custom of many. At the same time the problem has not been basically nor sufficiently elucidated experimentally, and the results are not constant nor consistent enough clinically to put the question on a firm foundation of fact. One is torn between the intricacies of theory and the results obtained in practice.

If you do not believe in vaccines you either have no interest in them or at most only a passing fancy. If you do believe in them you may be a hyperenthusiast. Both of these positions are untenable. I believe that the midground

between two extremes is always a safe place to work in, and this is certainly true for the vaccine problem. In other words, stay in the fairway, keep out of the rough but keep playing and do not pick up.

What is a vaccine? A vaccine is a buffered solution of immunogenic or vaccinogenic bacterial substances, bacterial bodies, toxins, phages, and filtrates prepared in such a way that the greatest chance for obtaining and maintaining specific antigenic or stimulus body is preserved intact. To begin with, there are all manners of vaccines, prepared by all sorts of methods and so with great variation of antigenic substances. If possible, the properties of the antigen or vaccinogenic substance or substances, whether or not of protein nature, must be accurately determined and measured with better methods for the recognition and dosage devised.

At the present time I am assuming that the isolation of a single organism from a given lesion, as for example, the hemolytic white or yellow staphylococcus from a cutaneous abscess (boil or furuncle), in pure culture, is the etiologic stimulus for that infection, and that its combat may be accomplished by the production of antibodies produced in the body which are more or less specific against it. It is well recognized that a properly prepared vaccine, autogenous or stock, will effect prompt results in a large number of these cases. Against this there are many failures with recurrences. In these cases of failure, there is a chance for the study of the antigen which is probably quite specific or at least the conditions for specificity are quite apparent, and the effects of modifications of vaccine preparations can be nicely studied and the results interpreted.

In the case of multiple organisms in a flora, in which one or more may be the etiologic factor, the problem is more complicated but the same principles may be applied to each organism isolated as though dealing with a single organism infection. The best example for multiple types is the flora from the sputum and upper respiratory tract in pulmonic infections. Many excellent results are obtained in the use of vaccine in bacterial asthma and chronic bronchitis and bronchiolitis. In these cases I plant the material by light streaks on blood agar plates and select colony types in order of their quantitative growth. From these first transplants, salt solution preparations are made and sterilized, keeping each type separate. These usually average four, five, or six. Where a number of different sources, feces, urine, prostate, cervix, gingiva, are suspected as foci, isolations are made in the same way. This may amount to twenty or more individual preparations. The organisms to be used in the final vaccine are picked out according to the intradermic, allergic reaction of each. This reaction must amount to 50 per cent or more and is read immediately, within one-half hour, and again for delayed reaction in twenty-four hours. Only those organisms showing definite cutaneous allergic reactions are considered for use. This principle of restricting the use of possible etiologic bacteria to only those which give positive allergic intracutaneous reactions may eventually be shown to rule out some organisms having a vaccinogenic substance but my own results during the past year have been improved I think because of this procedure.

A vaccine, therefore, must be started if possible from the actual specific etiologic organism. It must be prepared, utilizing bacterial bodies and soluble bacterial products in such a way that their antigenic or vaccinogenic factor is

altered in the least possible degree. No matter how desirable a living organism might be from the standpoint of its vaccinogenic value I believe it should be killed. I am still heat-minded, using the smallest amount for the least time with the addition of tricresol. The final control from the finished ampoules must be sterile, using the same medium or media as in the original isolation. I realize the possible value of attenuated forms but I still fear their potential possibilities.

The dosage of a given vaccine varies with each one given. The present trend is to start with very small doses, at least 0.1 c.c. and increase in 0.1 c.c. stages keeping the dose just short of local or general reaction. I believe that general reactions (malaise, some headache, slight fever, and an increase in focal symptoms) are not only to be desired but are essential if the fullest degree of immunologic factors are to be obtained. I pay very little attention to the local reaction unless it is severe, when I delay the next dose for a day or two, but I do not consider a maximum dose until a general reaction has repeated itself at least two or three times. The local reactions are so uncertain, may be severe with the first dose or not at all with much larger doses, that they cannot be used as an intelligent guide to dosage. General reactions, on the other hand, are quite definite and appear a few hours after injection. I try to get a result comparable to that of a typical antityphoid reaction.

Bearing in mind my method of bacterial selection and preparation with my reaction to dosage, I pay no attention to bacterial counting in the original vaccine but always use heavy suspensions, so that my vaccines are always milky cloudy as compared to the light almost watery preparations of most laboratories.

Vaccines are wonderful adjuncts but are not cure-alls. They must be given with the definite purpose of raising the immunologic bodies against a given infection, if our theories on immunity have any basis, in fact, but that infection must be reduced or eliminated wherever possible by all other means available. A great many of the indifferent results and failures can be charged, not only to the nonspecificity of the vaccine used, but to the fact that vaccines alone are incapable of combating the conditions of infection.

The selection of cases for the use of vaccine therapy is also of great importance. In all types of infection, direct or focal, a vaccine is indicated, and good results may be expected in a high percentage. This percentage of results is improving as the selection of cases is more carefully made, as the vaccines are more intelligently prepared, as the dose is more adequately administered, as the originating foci are more thoroughly eliminated, and as direct chemotherapy where indicated is more specifically applied. Thus, it seems to me the vaccine situation of today is summed up.

Shall vaccines be given intracutaneously, subcutaneously, intramuscularly, or intravenously? The intracutaneous method will undoubtedly give vaccine results but the dosage must be smaller. I am not in a position to recommend it exclusively at this time. The subcutaneous method has been and is largely preferred, but for the past year or more, I have been using a deeper intramuscular injection. This can be done with much less annoyance to the patient, less local reaction, and with as efficient vaccine results. It seems to me that eventually the intravenous route will be the method of choice but as long as bacterial bodies are used, they might become embolic and might initiate thrombosis.

The giving of a dose of vaccine is an art. Good, new, sharp needles are essential, the syringe, of course, must be sterile. The proper amount of vaccine is drawn into the syringe after sterilizing the top of the bottle with alcohol, the surface of the arm or leg is thoroughly cleaned with sterile gauze, alcohol and dried. The area selected over a muscle, in the arm the triceps, is gently held with the left hand and the needle quickly plunged into it. The contents of the syringe are injected as quickly as possible, the needle withdrawn and the area gently massaged with a sterile sponge. The giving of a small dose should be accomplished in less than a second and even one cubic centimeter in two or three seconds. This rapid method is approved and appreciated by the patient. As expressed by Kolmer, I think the result of vaccine dosage is greatly enhanced in the hands of those with increasing experience.

CONCLUSIONS

The position of vaccines, theoretically, experimentally, and clinically is today one of imbalance.

There has been too much accomplished in the past to disregard them entirely and a middle ground is an acceptable attitude.

A vaccine is defined as a vaccinogenic solution having the power of producing specific antibodies in vivo.

Vaccines prepared from a multiple flora are selected by me exclusively on their quantitative appearance in culture and their allergic cutaneous reactions.

Vaccines must start out with the study and isolation of specific etiologic organisms.

Vaccines must be prepared in such a way that the vaccinogenic factor is as nearly unaltered as possible, but I am not in favor of using any solution where a viable organism has any chance of becoming virulent.

The present trend in dosage is to start with small amounts, 0.1 c.c. increased in 0.1 c.c. stages and given every other day over a long period. Each individual case is an entity and for this reason the best results are obtained after experience.

In the preparation of a vaccine, I pay no attention to the bacterial count but make heavy suspensions and vary the dosage according to general reactions.

The desirability of general reactions for immunity producing purposes is stressed.

Vaccines are wonderful adjuncts but not cure-alls.

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EXAMINATION FOR PATHOGENIC FUNGI*

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FUNGI are those plants of the Thallophytes which are devoid of chlorophyl and must, therefore, depend for their food upon vegetable or animal tissues. In the examination of any material for fungi, there are two modes of attack: one is the direct examination of the material, and the other is the cultivation of the organism from the material. In the first case the material, either stained or unstained, may be examined with the aid of the microscope. In general wet (unstained) preparations are preferred to stained ones, because the morphology of this group of organisms is so characteristic in the unstained condition. Pus cells and fibrin, in exudates, and the tissue cells in tissue sections generally obscure the field, therefore the material is best placed on a slide and a few drops of antiformin, 20 to 40 per cent sodium hydroxide, or alcohol sodium sulphide[1] is added to the material on the slide, covered with a cover slip and examined. If scrapings from the skin are to be examined the alcohol sodium sulphide will be found to be superior to the antiformin or the sodium hydroxide. The sodium sulphide will clear the specimen in a few minutes, where the time necessary to obtain a satisfactory slide with the hydroxide will require several hours in contact with the solution. In either case I have found it profitable to examine the slide the next morning, adding water, if necessary, to replace the loss due to evaporation. Evaporation may be retarded by placing the slide in a Petri dish containing moist filter paper. It must be borne in mind that certain fungi are dissolved in sodium hydroxide.

In the normal and abnormal tissues of man and animals there are fibers very similar to the filaments of mycelium. The mycelium reported in sections of the spleen in cases of splenomegalia is an example of this.

NOMENCLATURE

When the mycelium is not divided into cells by partitions, it is said to be continuous, nonseptate or without septa. When the mycelium is divided into cells, it is said to be septate or noncontinuous.

Cells containing many nuclei are known as coenocytes.

Hyphae are special branches which bear spores. The simplest forms of spores are bud-like outgrowths on the mycelium and are called gemmae. This is well illustrated in the yeasts. The mycelium may be cut off by partitions, and the protoplasm inside gathered into a mass which has a thickened wall known as chlamydospores. Spores which are cut off from the tip of hypha are known as conidia or conidiospores, and the hypha bearing them is a conidiophore. Spores in chains are said to be catenulate, due to the development of one spore below another before the elder spore is cast off. Conidia composed of one cell are described as being simple, while those composed of two or more cells are compound.

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Spores may be contained loose inside of swollen tips of hyphae. These spore cases are sporangia and the hyphae are sporangiophores. The Mucors are of this type. The ascus differs from the preceding in that the number of spores is generally of a definite number, eight being the most common, although they may be lesser or greater; they are of the order 1, 2, 4, 8, 16, 32, etc. The asci may be naked or covered, scattered or collected into groups. When naked the cup or disk which bears the ascus is known as an apothecium; when covered the fruit is a perithecium.

Sexual spores are formed by the fusion of sexual elements known as gametes. The Oömycetes reproduce in this manner. Arthrospores are the fragmentation of the mycelium and are also known as Oidia. Oidium lactis is an example. Aleurispores are attached to the mycelium and differ from conidia merely in their place of attachment. Hemispores are structures which appear to be a transition between the yeasts and the oidia, and conidia; some of the cells of the mycelium develop a swollen structure which later breaks up into a series of spores resembling arthrospores. Aleurispores differ from conidia in that they are not set free when mature, but are only liberated when the mycelium that forms them disintegrates. These structures are analogous to lateral chlamydospores.

DETERMINATION OF MORPHOLOGY

The mycologist identifies fungi by morphologic methods and for this method Petri dishes and slide cultures are to be preferred to those of the culture tube. The growths on the medium in the Petri dishes are first examined with the unaided eye, then with a hand lens. A lens of 4× is very good for this work. Following this the growth may then be examined with the 16 mm. objective, using the microscope. Considerable information may be obtained by inverting the dish, without removing the cover, and examining with this objective. This keeps the culture free from contamination but the aerial hyphae cannot be examined with the clarity that is possible with the cover removed. The 4 mm. objective may be used to a limited extent, with the cover removed. By these methods the fruiting organs may be examined with considerable detail. The finer details of the structure may be seen by removing some of the growth to a slide, mounting in water, applying to cover slip and examining with the higher powers. By these methods the structure of the mycelium (whether it is septated or not), the structure of the sporophores, the position of the spores, together with their shape and number, the presence or absence of chlamydospores, and other morphologic characters may be studied. Pleomorphism of fungi due to variation in culture media and the age of the culture should always be kept in mind.

CULTIVATION OF FUNGI

The best medium for the isolation of fungi, provided the sought-for species will tolerate the acidity, is one made as follows: one per cent peptone and one and one-half per cent agar in water, distribute among culture tubes in 10 c.c. quantities, plug with cotton and autoclave. Prepare an aqueous solution of 50 per cent dextrose and 5 per cent tartaric acid and autoclave. When required for use melt the agar and add 1 c.c. of the dextrose, tartaric acid mixture. This

gives a concentration of about 5 per cent dextrose and 0.5 per cent tartaric acid. The reaction is about P_H 4. Most fungi will grow luxuriantly on this medium, while the bacteria are almost completely retarded.

Sabouraud's medium is extensively used for the cultivation of fungi. This medium contains 1 per cent peptone, 4 per cent crude maltose and 1½ per cent agar. Sabouraud's "proof agar" is made by using a particular brand of peptone and crude maltose. For most work any brand will suffice and dextrose may replace the maltose.

Wort and aqueous extracts of numerous vegetables are used to grow fungi, as are also the vegetables themselves prepared after the manner of the potato culture used in bacteriology.

THE MORE IMPORTANT FUNGUS DISEASES

Actinomyces: The majority of these infections present grains in the pus. When grains are crushed on a slide and stained with one of the aniline dyes, or by Gram's method, they will be seen to be made up of fine threads, generally gram-positive. A great many members of this genus are aerobic, but some are anaerobic; therefore, in plating the cultures should be grown aerobically and anaerobically. Growth is generally slow. The organism may be demonstrated in the tissues by histologic methods, using eosin and hematoxylin, or other stains. The characteristic club forms are seen in the tissues (Fig. 13).

Trichosporosis: This is due to a Trichosporum. Masses of mycelium grow as little hard knots on the hairs. These knots give off a rattling sound when the comb is run through the hair.

Blastomycosis: Doubly contoured, budding cells may be demonstrated in the pus either in potash solution or by staining. Histologic sections, stained with eosin and hematoxylin, will reveal the causative organism in the miliary abscesses (Fig. 15). In some cases it may be necessary to make a number of sections, ten or more, to demonstrate the causative organism; in other cases each section may contain large numbers of the parasite. The organism (Oidium gilchristi) may be grown on the common laboratory agars. It produces a white fluffy growth made of mycelial threads (Fig. 14).

Pityriasis Versicolor: Scrapings of the skin are placed on a slide, potash solution or sodium sulphide solution added and a cover slip laid on top of the liquid. After a few minutes with the sulphide solution (longer with the potash) the clusters of spores and the short mycelial threads will be seen (Fig. 11).

Thrush: Slide preparations from the patches stained by Gram's method will show gram-positive budding forms. If this material is plated, the colonies may be isolated by identifying them by use of the 16 mm. objective of the microscope, as described in a previous section of this paper. The colonies have a greenish cast, are somewhat circular and are composed of globular elements. A pure culture planted into dextrose broth, after a few days show not only the budding types but also a mass of long branching mycelial threads, many of which have budding spores. To determine if these forms produce ascospores, it will be necessary to plant some of the growth on a plaster of Paris block. This block is made by mixing plaster of Paris with water, pouring into a paper mold, and allowing it to harden. When this has occurred, remove it from the mold

and place it in a deep Petri dish or test tube. Water or 0.1 per cent peptone solution is added to thoroughly moisten the block, but the solution must not cover the surface. The moistened block is then sterilized in the autoclave. Growth from a young culture is generously spread over the surface of the block. McKelvey's medium may be used in place of the plaster of Paris block. This is

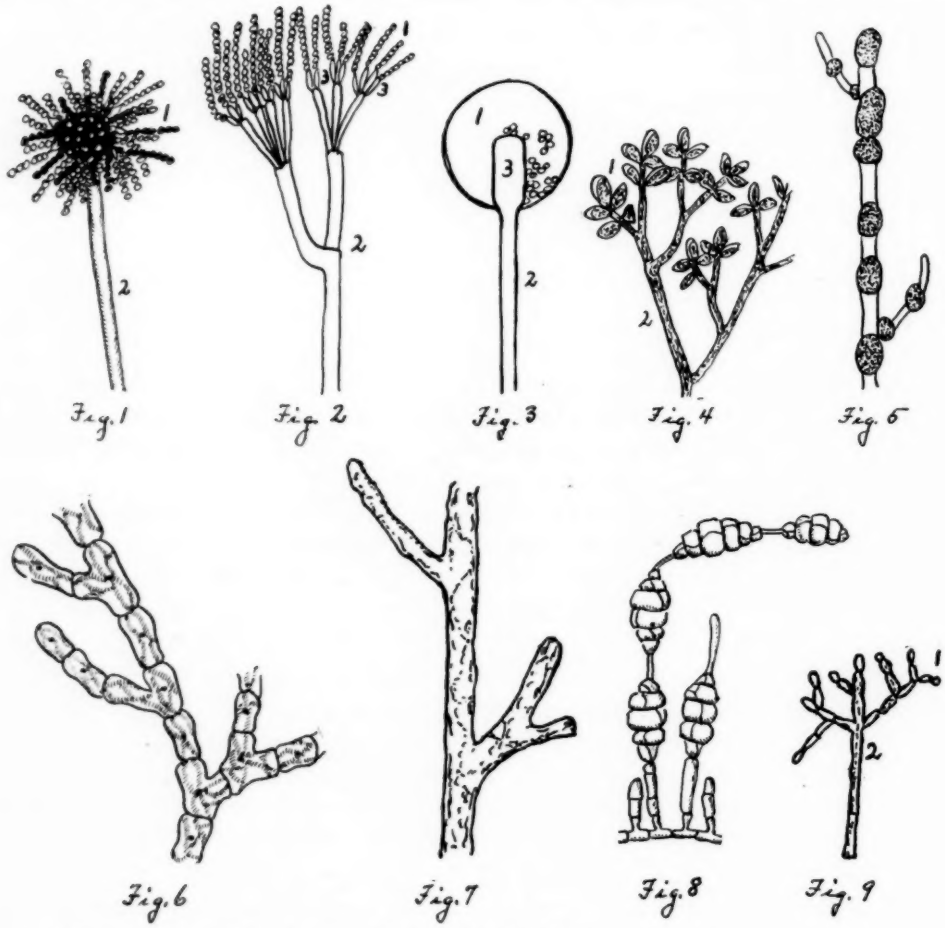

Fig. 1.—Aspergillus. *1*, conidia; *2*, conidiophore.
Fig. 2.—Penicillium. *1*, conidia; *2*, branching conidiophore; *3*, sterigmas.
Fig. 3.—Mucor. *1*, Sporangium; *2*, sporangiophore; *3*, columnella.
Fig. 4.—Trichosporium. *1*, conidia; *2*, conidiophore.
Fig. 5.—Chlamydospores.
Fig. 6.—Septate mycelium.
Fig. 7.—Nonseptate mycelium.
Fig. 8.—Spores of Alternaria.
Fig. 9.—Cladosporium. *1*, conidia; *2*, conidiophore.

made by adding plaster of Paris to carrot infusion agar, which is then tubed, autoclaved and slanted as with any other agar culture medium.

The above methods may be used for moniliasis in general. The yeast-like bodies will be seen on microscopic examination; the material may then be plated, the organisms isolated in pure culture and identified. So much controversy has arisen concerning the species of the genus Monilia, that it seems best not to include them in this paper.

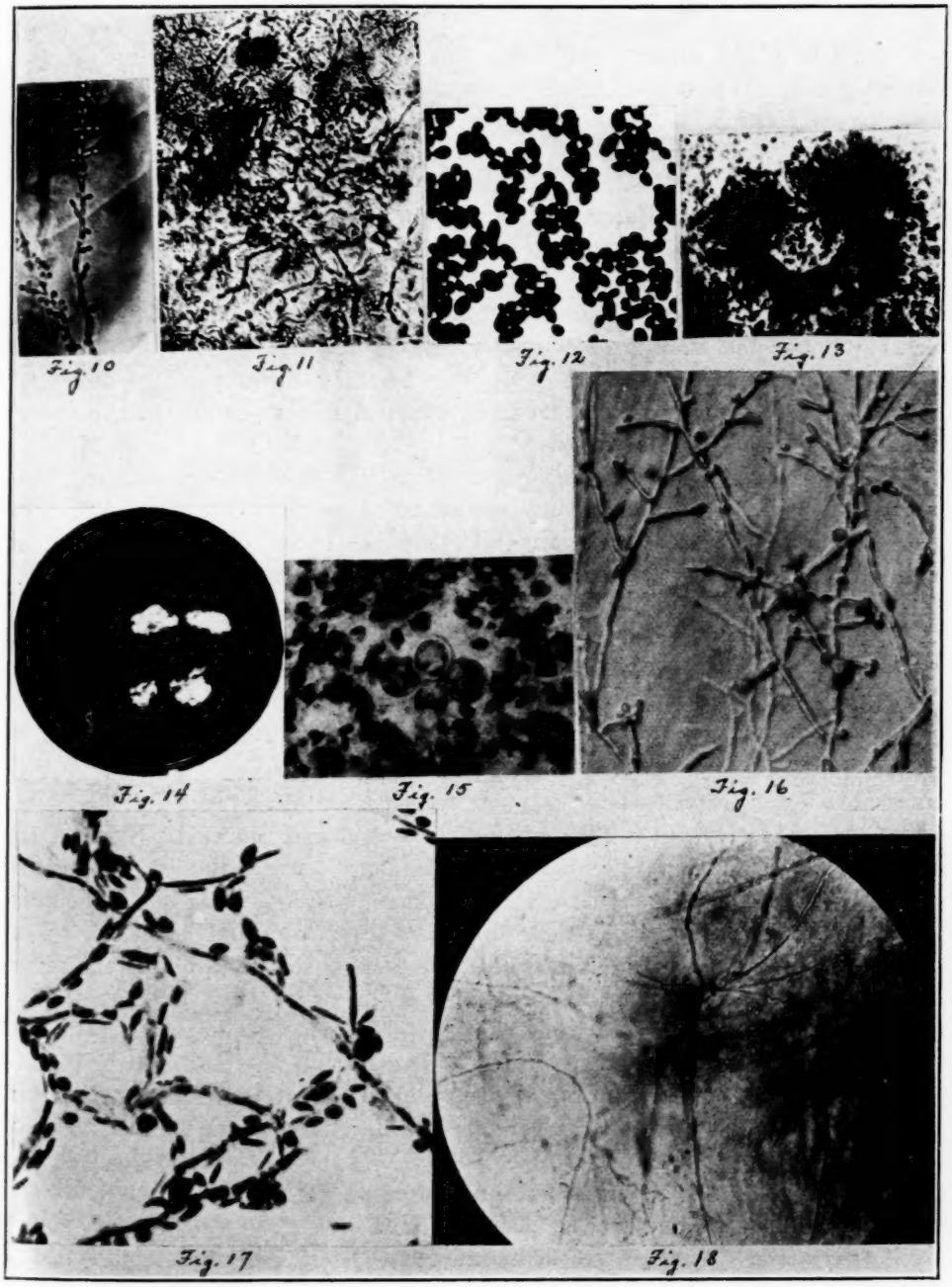

Fig. 10.—Monilia. Budding mycelium.
Fig. 11.—Malassezia furfur from Pityriasis versicolor. Wet preparation showing mycelium and spores.
Fig. 12.—Monilia. Budding forms (blastospores). Stained preparation from agar slant.
Fig. 13.—Actinomyces in tissue. Stained.
Fig. 14.—Oidium gilchristi. Growth on agar.
Fig. 15.—Oidium gilchristi in tissue. Stained.
Fig. 16.—Sporotrichum schenckii. Growth on agar. Unstained. 4 mm. objective and ×10 ocular.
Fig. 17.—Sporotrichum schenckii. Stained preparation from agar slant.
Fig. 18.—Trichophyton in skin. High dry. Sodium hydroxide preparation. 4 mm. objective and ×10 ocular.

THE BRONCHOMYCOSES

Diseases of the bronchi and lungs may be due to, or associated with, the following fungi: Those resembling yeasts, i. e., the budding forms, Coccidioides, Endomyces, Monilia, and Saccharomyces; those of the slender filamentous type, Actinomyces, Anaeromyces and Vibriothrix; those of the larger type, Hemispora and Oidium; those with characteristic fructifications, Acladium, Acremoniella, Alternaria, Aspergillus, Mucor, Penicillum, and Sporotrichum.

The descriptions of the above genera follow:

Coccidioides: Asci present; vegetative cells form a well developed mycelium. The asci contain a large number of spores; characters intermediate between Saccharomyces and Monilia.

Endomyces: Mycelium well developed; budding forms present; asci four spored formed asexually; reproduction by external spores, ascospores and spores situated inside the mycelial tubes.

Saccharomyces: Vegetable cells globose, ellipsoid, ovate, pear-shaped, etc., reproducing by budding and remaining attached in short, simple or branched pseudomycelial groups, which may later separate; asci globose, ellipsoid, or cylindric, 1 to 4 spored (typically 3 to 4 spored), single or in chains; ascospores globose to ellipsoid.

Anaeromyces: Anaerobic, gram-positive, nonmotile, branching diphtheroid bacteria; colonies moist.

Vibriothrix: Mycelium polymorphic; bacillary, vibrio-like, sprillum-like and at times clubbed. Globose or pear-shaped bodies, which vary in size, may be present. Generally motile; gram-negative; generally aerobic and facultative anaerobic, a few are anaerobic; grows on common mediums.

Hemispora: Mycelium thin, hyaline, branched and septated. Some of the mycelial filaments show a cushion-like structure termed the protoconidium, which later becomes segmented. These segments are known as deuteroconidia.

Oidium: Forms in which the hyphae are often long and branched, terminating in spores known as Oidia, which result from the septation of the mycelium. Budding forms are at times included in this genus (Fig. 15).

Acladium: Accessory fructifications absent; hyphae abundant, more than 1 micron in diameter; the hyphae are pale, elongated, septate and branched, without true conidiophores; sporophores unbranched; sporogenous apparatus but little differentiated from the mycelium; pseudoconidia borne on the walls of the mycelium.

Acremoniella: Mycelium septate, abundant; a dark brown spore, 7.7 to 9.7 microns in diameter, terminal on each conidiophore.

Alternaria: Conidiophores in bundles, erect, short; conidia septate in two directions; conidia in chains (Fig. 8).

Aspergillus: Conidiophores unbranched, rise from an enlarged cell of the vegetative mycelium; terminal portion swollen; conidia borne on sterigmas (Fig. 1).

Mucor: Sporangiophores arising singly, without stolons; spores borne in sporangia; mycelium septate (Fig. 3).

Penicillium: Conidia on distinct conidiophores, which branch at the tip; conidia 1 celled; neither conidia nor hyphae smoky or dark in color when young (Fig. 2).

Sporotrichum: Mycelium septate and branched; conidia borne laterally or terminally from all parts of the mycelium; true conidiophores absent; colonies may be white or colored (Fig. 16).

MYCOTIC DISEASES OF THE NERVOUS SYSTEM

Abscesses containing yeasts or yeast-like organisms have been found in the brain and spinal cord in patients with a generalized infection of these fungi. A number of cases have been reported of meningitis due to Torula histolytica. This organism appears in the tissues as yeast-like cells, surrounded by a thick capsule. In culture the growth resembles that of the yeasts, macroscopically and microscopically. No asci are formed and no gas is produced from any of the carbohydrates ordinarily used for fermentation tests.

MYCOTIC DISEASES OF THE ORGANS OF SPECIAL SENSES

The following fungi have been reported as the etiologic factor in diseases of the eye: Actinomyces, Blastomyces, Monilia, Oidium, Aspergillus, Penicillium, Sporotrichum, and Glenospora. The genus Glenospora is defined as mycelium composed of abundant hyphae, septate and branched; conidiophores absent; hyphae pale or dark; small aleurispores become dark and are borne on the sides and tips of the hyphae.

Aspergillus infection in man is commonly located in the ear. Monilia, Mucor, and Saccharomyces infections of the ear have been less frequently reported.

MYCOSES OF THE GENITOURINARY SYSTEM

Primary or secondary infections of the urethra in which a discharge is generally present may be due to one of the following: Actinomyces, Monilia, Oidium, Torula, or Saccharomyces.

The most common fungus associated with vaginitis is the genus Monilia. A number of cases of vaginitis due to this genus have recently been reported. Other genera reported are Alternaria, Aspergillus, Cladosporium, Hemispora, Penicillium, Torula, and Vibriothrix. Most of the latter group may be mere saprophytes.

INTESTINAL MONILIASIS

Patients who harbor Monilia in their intestinal tracts are not uncommon. Sprue has been said to be due to this genus. The Monilias may be demonstrated very readily by planting the suspected material on dextrose-tartaric acid agar.

MYCOTIC DISEASES OF THE SKIN

Mycotic diseases of the skin may be divided into two groups: (1) Those which produce an inflammation due to the invasion of the tissues; and (2) those which produce no true infection, but merely a saprophytic growth on the epidermis or hairs (Malassezia belongs to this group). The first group is known as the dermatophytes or dermophytes.

The dermophytes are characterized by a branched, septate mycelium, usually producing two or three forms of conidia in cultures; arthrospores, aleurispores and spindles (fuseau) are produced.

The Dermophytes may be classified as follows:

A. No conidia in cultures, Endodermophyton.

B. Conidia of one kind:

1. Conidia simple, globose to subglose
   a. Conidia in clusters, Malassezia
   b. Conidia borne at tip, Montoyella
   c. Conidia borne on the walls or sides, Pinoyella
2. Conidia fusiform and septate, Epidermophyton.

C. Conidia of two kinds:

1. Aleurispores and arthrospores present, Trichophyton
2. Aleurispores and spindles present, Microsporon
3. Aleurispores, arthrospores and hypae with dichotomous subglobose or club-shaped apical branches, Achorion.

The above genera are summarized below.

Montoyella: The members of this genus have two kinds of mycelium, the one slender, branching and segmented, the other broader with numerous intercalary chlamydospores. From the broader filaments are borne delicate hyphae, which terminate in globose and pear-like conidia.

Pinoyella: Mycelial filaments and spores in the lesions, and conidia-bearing hyphae in cultures. The spores are situated on the walls or sides of the tubes. No perithecia or asci present.

Malassezia: In man, mycelium broken into septate segments, with T-shaped or budding extremities. The hyphae bear round or oval conidia, which may be solitary or in clusters, and may be smooth or with radial, longitudinal or spiral marks. This organism has not been cultivated on artificial media (Fig. 11).

Epidermophyton: Mycelial filaments and spores present in the lesions and with pluriseptate spindles present in the cultures; hairs or hair follicles are not attacked; grows in the superficial layers of the epidermis. Spiral hyphae, which are present in most species of Trichophyton, are absent. There are no perithecia or asci present.

Microsporon: Members of this genus produce mycelial filaments and spores in the lesions. These spores are small, globose, and about 2 to 3 microns in diameter. In cultures hyphae-bearing sessile conidia and septate or nonseptate fusiform bodies may be seen. Perithecia and asci are absent.

Trichophyton: This genus is characterized by the formation of very distinct arthrospores in the mycelial filaments. These are present in the lesions and in cultures. There are no fusiform bodies present (Fig. 18).

Achorion: Mycelial filaments and spores are present in the lesions. In cultures, hyphae are present, which bear conidia laterally and at the tips. Fusiform bodies are also present in the cultures; these bodies are in the form of club-shaped terminations of the filaments. Asci and perithecia not present.

Endodermophyton: The members of this genus grow between the superficial and deep layers of the epidermis. Mycelial filaments and spores are pres-

ent in the lesions. No conidia are formed in cultures. No spindles, perithecia or asci present. To cultivate this fungus, treat the scales with alcohol for five to ten minutes, wash with sterile water and place one scale in each of several tubes of dextrose broth. Growth appears in about a week. In about three weeks the fungus may be transferred to dextrose agar slants.

CLASSIFICATION OF FUNGI

The classes of the subdivision Fungaceae which are of medical interest are the Phycomycetes, the Ascomycetes and the Fungi imperfecti.

The Phycomycetes produce a copious matted mycelium, which is nonseptate, and reproduce asexually by means of sporangia borne on columnellae.

The subclass zygomycetes reproduce either asexually (production of sporangia) or by isogamy (two similar but sexually different cells conjugate, which on fusion form a zygospore).

This subclass is divided into two orders: Those with several asexual spores on sporangia (Mucorales), and those with solitary spores (Entomophthorales).

The order Mucorales contains the family Mucoraceae. In this family are three genera: Mucor, without rhizoids; Rhizomucor, with rhizoids and unbranched aërial hyphae, and Rhizomucor, with rhizoids and ramified mycelium.

The class Ascomycetes contains the following genera: Saccharomyces, spores smooth, spore membrane single; copulative process absent; rudimentary mycelium present, with transverse septation; alcoholic fermentation produced. Endomyces, mycelium well developed, ramified or not, simple or septate; budding cells present; asci four spored.

KEY TO SOME OF THE GENERA OF FUNGI

A. Mycelium white or bright-colored; conidia 1-celled, hyaline or bright-colored, globose to ovoid or cylindric.
  1. Hyphae very short or obsolete, or little different from the conidia
    a. Conidia in chains, produced by blastospores arising on the hyphae; globose or elliptic; hyphae branched MONILIA
    b. Conidia in chains, produced by arthospores; ovoid to elliptic; hyphae branched OIDIUM
  2. Hyphae elongate and distinct from the conidia
    a. Conidia borne in heads
      (1) Conidiophores inflated at apex; conidia in chains at the tip; globose to ellipsoid ASPERGILLUS
      (2) Conidiophores not, or but slightly, inflated; conidia not in mucus; globoid; in whorls at the tip; whorls unequal PENICILLIUM
    b. Conidia borne more or less irregularly on simple or branched but not inflated or whorled hyphae; conidia smooth or scarcely roughened
      (1) Conidia borne on the walls or sides of the mycelium; conidiophores simple or nearly so; conidia globose to ellipsoid ACLADIUM
      (2) Conidia borne at the tip and sides of the mycelium; conidiophores vaguely branched and of one kind; conidia globose to ellipsoid SPOROTRICHUM

B. Mycelium dark or smoky.
  1. Conidia 1-celled, dark, or sometimes hyaline but the hyphae then dark; globose to oblong
    a. Hyphae very short or scarcely different from the conidia; conidial chains breaking up readily TORULA
    b. Hyphae manifest and distinct from the conidia

(1) Conidia in chains on the branches, ovoid or globose; conidiophores erect, dendritically branched and septate; not spirally twisted HORMODENDRUM
(2) Conidia not in chains
(a.) Conidiophores branched; all hyphae more or less creeping; conidia smooth and sessile TRICHOSPORUM
(b.) Conidiophores swollen at tip; conidio 4-8 superimposed, warted HEMISPORA
(c.) Conidiophores not swollen at tip
x. Conidia solitary, smooth, on short lateral branches; hyphae forming a crust GLENOSPORA
y. Conidia solitary, smooth, terminal; sterile hyphae present, without bristles ACREMONIELLA
2. Conidia 2-celled; conidiophores definitely differentiated; conidia not in terminal heads; conidia in chains and of one kind; hyphae not inflated CLADOSPORIUM
3. Conidia many-celled, with both longitudinal and transverse septa ALTERNARIA

In conclusion the reader is referred to the works of Henrici, Clements and Shear, Brumpt, Sartory and Castellani for more extensive information.

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BACTERIOLOGY OF PUS*

By John F. Norton, Ph.D., Detroit, Mich.

PUS is formed as the result of an accumulation of leucocytes, largely the neutrophils, which have migrated from the blood vessels into irritated tissue. Necrosis and liquefaction of the cells usually follows. A positive chemotactic action is responsible for the accumulation of leucocytes. This chemotaxis is due to the presence of cellular constituents, possibly protein in nature, which have been set free by injured cells. These may be the fixed tissue cells of the body or may be of microbic origin. Pus does not therefore, necessarily contain bacteria but in an overwhelming proportion of instances in which this material is sent to the laboratory for examination, bacteria of some variety are found. It may however be difficult to demonstrate the presence of bacteria in pus from the cases of certain diseases after therapeutic measures have been instituted.

The variety of bacteria encountered in pus, is great. Indeed, a complete discussion would entail the preparation of a textbook on pathogenic bacteria. It is, therefore, possible to give here only an outline of methods in most common use, and to refer to the microorganisms most generally encountered. Regardless of the source of material, whether from a running ear, a case of empyema, furunculosis, or meningitis, certain definite procedures can usually be followed with the introduction of proper variations dependent on the results obtained as the laboratory worker proceeds. It is possible to give in this brief discussion, only the most outstanding characteristics of the groups of bacteria referred to,

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these characteristics being chosen because of their value to the clinical laboratory. For exact identification of bacterial species, reference must be made to the literature.

The proper collection of material is essential for a satisfactory laboratory examination. Skin contaminants must be rigidly excluded. Too great emphasis cannot be placed upon this point, if the laboratory worker is to give the clinician a true picture of the bacteriology of the material submitted.

The first step in laboratory examination of pus is, under all conditions, the *preparation and examination of a proper smear* on a microscope slide. Care should be taken to make thin film preparations. A stain according to Gram's method gives the most valuable indication of any single stain but will not, of course, detect the tubercle bacillus. Hence it is extremely desirable for the laboratory to be furnished with accurate information concerning the source of the material. Various modifications of the original Gram method have been introduced.[1] Our experience has been that careful attention to the details of the technic is fully as valuable in yielding consistent results as any modification of the solutions employed. This is particularly true in regard to the alcohol (or acetone) decolorization. In "old" pus the Gram stain may be uncertain. In many laboratories, the methylene blue stain is used for the detection of gonococci. While this is reasonable under special conditions where the clinician and the laboratory work in close cooperation, it is not to be recommended. Furthermore, it is highly desirable to make a Gram stain if organisms resembling the gonococci are seen in the material submitted, so little is gained by the use of methylene blue. For pus from tubercular conditions, the Ziehl-Neelsen method, or some of its modifications, is in general use. The methylene blue stain may be used for detecting organisms resembling diphtheria bacilli while either methylene blue or gentian violet are useful for staining the spirochetes and fusiform bacilli characteristic of Vincent's infection.

It is undesirable that a laboratory report based upon the microscopic examination of a direct smear should record the name of the suspected organism. A possible exception to this is in the examination for tubercle bacilli and here the exception can be made only in material which is not likely to contain other "acid resisting" organisms. Undoubtedly, in many instances the name of a bacterium recorded on a laboratory report as a result of direct examination is correct, as for example, in vaginal or urethral smears in gonorrhea, in spinal fluid pus in epidemic meningitis, in staphylococcus infections, and in Vincent's angina. However, so many exceptions occur that such reports can be regarded only as preliminary and unscientific. Organisms of the N. catarrhalis group may be present in supposedly gonorrheal pus, or gonococci or N. catarrhalis might be the cause of meningitis, although it must be admitted that this difficulty rarely occurs.

It is sometimes claimed that the pneumococcus may be distinguished from Streptococcus viridans by direct examination. We do not believe that this can be done. Indeed it should never be attempted. The streptococci are highly pleomorphic and oftentimes closely resemble the typical lanceolate pneumococcus. With the present possible use of antipneumococcic serums in Type I and II infections, it is all the more necessary not to hazard a guess on the basis of micro-

scopic appearance. Morphologic variations in bacterial species have been definitely established. Residence in body tissue, living or necrotic, may accentuate these changes. In a fairly large series of cases of influenzal meningitis that have come to our attention, the organisms usually appeared in the spinal fluid as tiny gram-negative diplococci with only an occasional rod. In a few instances, rods predominated and in one case swollen cocci and rods were found. The laboratory should report the result of the Gram stain as quickly as possible, together with a statement concerning the appearance of the organisms found. In many cases this description will be sufficient for the purposes of the clinician.

Satisfactory identification of bacteria found in pus, can be made only as the result of biochemical and serologic studies. The isolation of the organism in pure culture is, of course, the first necessity. The culture medium of choice will depend to some extent on microscopic findings. It is impossible to pick one formula from the many hundreds available for media, and recommend it as the best under all circumstances. For all around work, except in cultivating tubercle bacilli and anaerobes, a meat infusion peptone agar containing one-half to one per cent of dextrose with a reaction of P_H 7.6 and containing about 5 per cent of defibrinated blood (the source of blood is in most instances immaterial) is to be recommended. This medium, in Petri dishes, will grow all the ordinary pathogenic bacteria encountered in pus, except as just noted. Better results are probably obtained if hormone broth is used as the basis of the medium but our experience has been that the extra work involved is not justified by the results. The medium is the one commonly recommended for culturing of meningococci from the throat. Our experience has been that it is excellent for the isolation of gonococci and that H. influenzae and H. pertussis, while not developing so rapidly as on some other media, can be grown satisfactorily. The main disadvantage of the medium is that some strains of streptococci fail to show as early hemolysis as when a more acid medium is used. If, therefore, the laboratory worker is certain that he is dealing with the streptococcus-pneumococcus group, the ordinary blood agar medium (P_H 6.8) gives earlier differentiation.

While certain differentiations can be made by the use of the ordinary so-called plain agar medium, such as between N. catarrhalis and the pharyngis siccus group from the gonococcus and the meningococcus, other media are of far more use in the clinical laboratory. Where material is from such a source that it is unlikely to contain more than one organism, semisolid agar will give excellent results. It does not, however, give any species differentiation such as is possible by the use of the blood agar plate above described. Many other special media are in use. Tubercle bacilli may be grown on a variety of media. When the organisms have been found in the direct microscopic examination, cultivation is not usually necessary unless it is desired to make a complete bacteriologic study of the case. It very frequently happens however, that the number of tubercle bacilli present in the material is so small that they cannot be found in the direct smear. Where there is any suspicion that these organisms might be present either guinea pig inoculation or culturing should be resorted to. Again, the medium to be used will depend somewhat upon the experience and ideas of the laboratory worker. Corper's medium, Petroff's medium, Petragagni's medium, Sweeney's medium, and others have been found useful. In our opinion,

the preparation of the specimen is of equal importance with the medium. Other bacteria must be eliminated, preferably by the use of acid (specimen and 3 per cent hydrochloric acid in equal volumes has proved successful in our hands), and more than one tube must be inoculated. If the guinea pig method is used, not less than two pigs are required.

Even if no bacteria are visible in the direct microscopic examination, it is still necessary to culture the pus. Either the blood agar plate above described, infusion broth, or a semisolid medium may be used. Anaerobic methods should also be employed if gangrene or tetanus is suspected. Brain broth or deep glucose agar are excellent general media for anaerobic work. A great variety of media are recommended to cover special circumstances.[2] All cultures should be incubated for not less than four days provided no growth appears within that time. Many mistakes are made in the identification of bacteria by the use of insufficient incubation periods. This is particularly true in testing the fermentation reactions of pure cultures. Delayed fermentation of carbohydrates as well as delayed reaction in milk are more commonly encountered than is sometimes supposed.

As indicated above, it will be possible to mention only the most commonly encountered pathogenic bacteria, and to give only brief descriptions of the various species. It is hoped to make these descriptions sufficiently adequate to cover the needs of most clinical laboratories. They are, of course, quite insufficient for scientific purposes or for detailed study of clinical cases. It is to be regretted that in so many instances, clinical necessity is a bar to accurate scientific work, but it must be admitted that with few exceptions, detailed laboratory examinations of bacterial cultures from pus do not give the clinician a better basis for therapeutic measures than a less detailed study. There are, however, some exceptions to this point of view. An attempt has been made to list in connection with the brief descriptions of bacteria, the source of material most commonly yielding the species described. It should be emphasized that the statements that follow refer only to pus.

The staphylococci are more frequently found in pus than any other group of bacteria. Among these, Staphylococcus aureus predominates. This is a gram-positive organism appearing in characteristic clusters on the microscopic slide. Irregular forms are sometimes encountered in pus. The organisms grow readily on all the common culture media and produce a golden yellow pigment. Dextrose is always fermented, with production of acid but no gas. Maltose, saccharose, and lactose are usually attacked. Acid is produced in milk. Gelatin is usually liquefied. On blood agar, many strains give a clear zone of hemolysis. Immunologically, the group is heterogeneous.[3] No group of bacteria, with the possible exception of the streptococci, is found in so many pathologic conditions in which pus is present. Skin conditions of great variety, including furunculosis and impetigo, subcutaneous abscesses, abscesses of various internal organisms, middle ear infections, osteomyelitis, and meningitis, are examples. They also commonly occur in mixed infections.

Streptococci are nearly as frequently encountered in pus as are staphylococci. They are gram-positive organisms occurring in pairs or chains, usually nearly spherical but not infrequently elongated. Certain strains have been

shown to be highly pleomorphic.[4] This group of bacteria are most easily isolated on blood agar. Various degrees of hemolytic activity are observed on this medium.[5] For practical purposes two types must be noted, those that produce a clear zone and those that produce a green coloration. Fermentation reactions are quite varied. Dextrose is always fermented, most human types ferment lactose, while various reactions are obtained with mannite, inulin, and other carbohydrates. Gelatin is not usually liquefied. Any classification on the basis of fermentation reactions is of little clinical significance.

The *hemolytic streptococci* are the more important of the two groups as differentiated on blood agar, because of their greater pathogenic power. No practical laboratory tests have been devised for distinguishing the varieties of these hemolytic organisms in relation to their specific pathogenicity, although many attempts have been made to do so. Neither serologic,[6, 7, 8] including agglutination, complement fixation or opsonic, reactions nor cultural[9, 10] tests have proved of value. This group is found frequently in abscesses occurring as complications of scarlet fever or other streptococcus infections, in ear infections, in meningitis, in bone infections, in tonsillar abscesses, in empyema, etc. The "green producing" streptococci are less frequently encountered. These organisms must be differentiated from the pneumococci (see below).

Pneumococci are frequently present in pus. These are gram-positive cocci usually seen in pairs but sometimes in short chains. The typical cells are elongated. It should be noted however, that in the examination of material from the body, atypical morphologic forms are more likely to be seen than the typical forms observed in pure cultures. Indeed, this is true of all varieties of bacteria. Pneumococci are easily isolated by mouse inoculation, in Avery's broth or on blood agar and produce a green coloration. The biochemical reactions are similar to those of the streptococci and are of little value in the clinical laboratory. The differentiation between a pneumococcus and the "green producing" streptococcus is important. In our opinion, neither colony characteristics nor morphology are sufficient for this purpose. The use of the agglutination or precipitin test combined with solubility in bile, are required. Solubility in bile is the generally recognized criterion for the separation of pneumococci and streptococci. However, we have isolated organisms of the pneumococcus-streptococcus group that are agglutinated by a pneumococcus type serum but failed to dissolve in bile. For this reason, reliance should not be placed solely on the bile test. Pneumococci are encountered in pus from ear infections, from meningitis, from peritonitis, in lung abscesses, etc. The typing of pneumococci may be important in cases where the clinician desires to use specific antipneumococcic serum.

Meningococci may be found in pus present in the spinal canal. They may also be present in the nasopharynx and the blood, but a discussion of these possibilities is outside the province of this paper. The meningococci are gram-negative biscuit shaped diplococci. While they are reported to be characteristically found in the leucocytes their presence outside of these cells should not be disregarded. Indeed, the extracellular position is characteristic of material from fulminating cases. Isolation may be made on the special blood agar medium already described or in semisolid agar.[11] It is important to work with a fresh specimen. Isolation after intraspinal injection of antimeningococcic serum is

difficult, although a stained preparation may reveal the organisms. Meningococci ferment dextrose and maltose. Fermentation reactions may be used to differentiate from other morphologically similar gram-negative cocci. For practical purposes however, identification of the meningococci can be more quickly made by the use of a polyvalent antiserum. Therapeutic serums with a relatively high agglutinin titer may be used for this purpose, but it should be noted that odd strains are sometimes encountered. There is no practical value in grouping the meningococci at the present time, but this statement may not hold when more knowledge is obtained concerning specific therapeutic serums.

Pus containing *gonococci* comes as a rule, either from the genital tract or the eyes. The organisms are infrequently encountered in spinal and other body fluids or organs. They are biscuit shaped, gram-negative, usually intracellular, and cannot be distinguished with certainty on purely morphologic grounds from other gram-negative diplococci. However, if the microscopic laboratory findings correlate with those of the clinician, it is unnecessary to proceed with further identification. In certain cases, complete laboratory identification may be extremely desirable. A great variety of media have been suggested for isolation of the gonococcus. The medium should be slightly alkaline and contain body fluids such as ascites, blood serum, or defibrinated blood. We have found the blood agar used for meningococcus work to be quite satisfactory. Gonococci ferment dextrose, but not maltose. The colony appearance on blood agar plates serves to differentiate them from many of the gram-negative diplococci. In order to obtain a successful isolation, fresh material must be used.

N. catarrhalis and the chromogenic diplococci are common inhabitants of the upper respiratory tract. They are infrequently associated with pyogenic conditions. N. catarrhalis can be distinguished from the meningococci by its ability to grow on an ordinary plain agar medium, and its inability to ferment carbohydrates. The chromogenic diplococci such as N. pharyngitidis sicci have stronger fermenting powers than the meningococci or gonococci. The extent to which the members of these two groups of organisms are concerned with pathogenic processes is problematical.

Members of the *colon typhoid group* are sometimes found in pus, B. coli being the most frequently encountered. As a group, they are relatively easy to identify. The gram-negative rods grow on plain agar and on various special media such as that devised by Endo. Gelatin is not liquefied. Fermenting properties vary. Members of the colon subgroup produce acid and gas from dextrose and lactose and sometimes from saccharose; the salmonella or paratyphoid group ferment dextrose with acid and gas production, but not lactose; the typhoid dysentery group ferment dextrose with the production of acid only. Colonies of B. coli of intestinal origin have a characteristic appearance on such media as Endo and eosin methylene blue. The other subgroups give a bluish white colony. The group as a whole is too well-known to require description here, particularly as the paratyphoid and typhoid subgroups are infrequently found in pus. Where possible, serologic identification should be made, including agglutinin absorption tests in the salmonella group. This last includes quite a variety of bacterial species and many groups, when studied on the basis of cultural and serologic properties.

The *Proteus group* is sometimes found in urinary pus. These organisms may also be associated with pyogenic processes other than those found in the urinary tract either in association with other organisms or as the supposed etiologic factor. The organisms are gram-negative, pleomorphic bacilli usually motile and do not ferment lactose but produce acid and gas from dextrose and saccharose. Most of them liquefy gelatin. Serologically, the proteus group is heterogeneous.

Considerable interest has been aroused during the past fifteen years over the disease known as *tularemia*. It is probable that the first human cases were eye infections reported by Wherry and Lamb.[12] B. tularense is rather difficult to grow, requiring either a medium containing egg yolk or blood or blood serum preferably in the presence of tissue; such carbohydrates as glucose, levulose, mannose, and glycerin fermenting with the production of acid. Final identification should be made by means of the agglutination reaction.

Bacillus pyocyaneus is found in socalled "blue pus" particularly from surgical dressings. Its virulence toward man is relatively low, apparently being somewhat greater for children than for adults. The bacillus is gram-negative, actively motile, grows well on plain agar, produces little or no acid from dextrose, liquefies gelatin and digests milk. It produces a blue-green pigment and a lysin toward red blood cells. It has been reported as one of the causes of middle ear infections and meningitis.

The *Friedlander bacilli*, while closely resembling B. aerogenes in cultural reactions can be distinguished by their morphology, by the presence of capsules and the mucoid growth on an agar medium. The organism is more frequently found in pus from upper respiratory tract infections than from any other type of disease although they have been reported in empyema, meningitis, cystitis, etc.

Organisms of the *diphtheria* group and the diphtheroids are rather frequently encountered in pus. However, they appear to be most often present as secondary invaders. In the latter case, either the diphtheroids or the very short bipolar staining organisms (similar to Wilson's Type D) are more likely to be found than are the morphologic types usually associated with diphtheritic infections (Wilson's Type C). Organisms of the diphtheria group present as secondary invaders are usually avirulent but there are exceptions to this statement. Their occurrence as the primary cause of pus producing infections cannot be lost sight of. Ears, thus infected and draining, may serve as foci for dissemination of the disease. Pleomorphism in the diphtheria group is too complex a subject to discuss here. Biochemically, the strains encountered are not particularly active. The true diphtheria bacillus produces acid from dextrose, galactose, and levulose and either no reaction or a small amount of alkali in milk. The pseudodiphtheria bacilli have a variety of biochemical reactions but in general the differences from the toxin producing organism are relatively small.

In culturing pus, the possible presence of *anaerobes* must not be overlooked. The tetanus bacillus is an example. In most instances relatively simple anaerobic methods are just as effective as are the more complicated ones. Growth will usually occur in a deep glucose agar shake culture or even in flasks containing broth. Probably the best medium for general use is one containing tissue, prefer-

ably brain.[13] B. tetanae belongs to the group of anaerobes which do not attack carbohydrates. Gelatin and coagulated protein such as blood serum, are usually slowly liquefied. A soluble toxin of high potency toward guinea pigs may be produced. Spores are formed usually near one end of a cell, giving a typical appearance resembling dumb bells.

The above mentioned groups of bacteria represent those most frequently encountered in clinical laboratory work, but as stated earlier in this paper, most any pathogenic bacterium may be found in pus. Attention should be called to the hemophilic, pasteurella, and lactobacillus groups. No mention has been made of material from pustules such as those found in smallpox. The possible presence of a filterable virus as the etiologic factor must always be considered even if known bacterial forms are present. Such pus, however, rarely finds its way to the clinical laboratory, perhaps not as often as it should. At any rate, the laboratory worker examining pus should not have any preconceived notions as to what he will find. Mixed cultures occur frequently and it is sometimes difficult to determine whether the localized infection is due to a mixture or to only one of the bacteria isolated. Even detailed studies do not always lead to a satisfactory conclusion concerning the etiologic agent.

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BACTERIOLOGIC EXAMINATION OF BLOOD AND SPINAL FLUID*

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THE problems of the clinical bacteriologist have been complicated in recent years by the introduction of a great variety of methods and mediums. Research has also tended to shake our faith in the fixity of bacteriologic types and suggests that the life cycle of microorganisms is not as simple as previously supposed. Regardless of these uncertainties the clinician is depending more and more on laboratory findings. A discussion of practical bacteriologic methods for the examination of blood and spinal fluid is here presented.

BLOOD

Collection of Specimen.—The sample is usually obtained from a vein at the bend of the elbow by means of a syringe and needle, or such special apparatus as the Keidel vacuum tube. The latter method is especially useful for the private practitioner who does not have adequate facilities for sterilizing apparatus, and where the transportation of the specimen by mail or messenger is necessary. For the hospital laboratory, however, the syringe and needle method is preferable.

The skin of the patient is sterilized with two coats of tincture of iodine and one coat of 95 per cent alcohol. Each coat of iodine should be permitted to dry before the application of the next coat.

Sterilization of Syringes and Needles.—As the sterilization and care of syringes and needles are usually inadequate, the following satisfactory method is given in detail:

The barrel of the syringe, the piston, and two needles are each placed in a pyrex glass tube closed at one end and open at the other. The tubes should be just large enough for the parts to be removed easily; for the larger syringes, they will have to be especially made. Cotton is placed in the bottom of each tube to prevent breakage, and after the parts have been inserted the open ends are stoppered with cotton plugs. The tubes are then tied together, wrapped in paper and retied, and sterilized in the dry sterilizer at 150° C. for two hours.

At the bedside, the instrument can be assembled easily with little risk of contamination. The cotton stopper in the tube containing the piston is first removed and if the latter has been encased properly, the head will easily slip into the hand. The stopper of the tube containing the barrel is next removed; the piston is slipped into the barrel and the two withdrawn together. In the same manner a needle can be attached to the tip of the syringe and after tightening the needle it is ready for use. If for any reason the blood cannot be taken immediately, the syringe can be replaced in one of the tubes with a minimal risk of contamination. After the blood has been taken, the syringe and needle should be washed in water before clotting of the residual blood takes

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place. Drying is quickly accomplished with alcohol and ether. The needle should be sharpened and tested for flaws. After determining that syringe and needles are in perfect condition they may be rewrapped and sterilized.

There are several advantages in this method: (a) The syringes and needles are actually sterile and dry. (Boiling for fifteen minutes or longer will not kill some contaminants.) (b) They can be readily transported in the sterile state. (c) They will remain sterile indefinitely and will always be on hand for immediate use. (d) The possibility of contaminating the apparatus at the bedside is reduced to a minimum. (e) Both syringes and needles prepared in this way will outlive, many times, similar equipment subjected to frequent boiling.

Routine Cultures.—Two mediums of special importance for routine culturing of blood are beef heart infusion broth and beef heart infusion agar. The details of their preparation are given in the article on the "Technic for the Isolation of Streptococci." The broth is made up in bottles or flasks, each containing about 50 c.c. The agar medium is conveniently placed in test tubes, 9 c.c. to each tube. Prior to use, the agar is heated in a water-bath until melted and allowed to cool in the air to about 40° C.; 1 c.c. of the patient's blood is then added, whirled until mixed, and poured into Petri dishes. The blood agar plate has several advantages. It gives a quantitative estimate of the number of bacteria in the circulating blood. Also, it frequently permits an early laboratory diagnosis. It is always well to make a culture in nutrient broth, as organisms that do not appear on the plates occasionally grow in this medium. In both cases, the amount of blood compared to the amount of medium should not exceed the ratio of 1 to 10. The optimum temperature for growth is 37° C., and the hydrogen ion concentration is slightly alkaline with a P_H of 7.4 to 7.6. It is usually advisable to incubate some of the cultures under anaerobic or partially anaerobic conditions as certain bacteria grow more luxuriantly in the absence of oxygen.

The blood agar plates and blood broth tubes should be observed daily for evidences of growth. As soon as the blood broth becomes cloudy, smears stained by Gram's method or with methylene blue should be made, as well as subcultures on blood agar plates and in blood broth tubes. Blood cultures should be observed for at least ten days and preferably fifteen to thirty days before being considered negative. There is a marked tendency to discard cultures at too early a date.

Gram-positive Cocci.—Staphylococci grow readily in all the usual laboratory mediums but for purposes of study, culturing on blood agar pour plates and in blood broth are the most satisfactory. In examining cultures attention should be given to their hemolyzing properties and their ability to produce pigment on solid mediums. Staphylococci, especially of the white variety, are occasionally seen as contaminants in blood culture work and a few colonies present on one plate and absent on others should make one suspicious of contamination. Staphylococci are identified by the characteristic appearance of their colonies on blood agar plates, by the fact that they are gram-positive, and by their tendency to form clumps instead of chains. Examination of smears for morphologic characteristics should be made from cultures in liquid mediums.

Streptococci may be recovered in blood cultures from patients suffering from a great diversity of diseased conditions. From foci of infection in the skin, tonsils, teeth, sinuses, and the middle ear, or from infected wounds, streptococci may invade the blood stream, producing a rapidly fatal septicemia, or chronic conditions, such as pleurisy, pericarditis, endocarditis, or arthritis. Primary cultures of streptococci grow delicately on artificial mediums and considerable care must be exercised both in obtaining them and in keeping them alive. The importance of observing blood cultures in liquid mediums over a long period is indicated by the positive results that have been reported after an incubation period of thirty days or more. Streptococci are differentiated from staphylococci by their characteristic growth in blood agar pour plates and their tendency to form long or short chains. Generally the individual cocci are smaller in streptococcal than in staphylococcal cultures.

Pneumococci are present in the peripheral blood of a large proportion of patients with lobar pneumonia and in the blood of some with bronchopneumonia. Positive cultures of this organism are sometimes obtained in complications of pneumonia, such as empyema, meningitis, mastoiditis, endocarditis, and pericarditis. The pneumococcus grows delicately in primary cultures but readily adapts itself to artificial mediums. Cultures are made in nutrient broth and in blood agar pour plates. Pneumococci are differentiated from streptococci by the bile solubility test, the former being bile soluble and the latter, bile insoluble.

Gram-negative Cocci.—Both the gonococcus and the meningococcus are occasionally recovered from blood cultures. Gonorrhea is sometimes complicated with septicemia and endocarditis, and in such cases the gonococcus is usually recoverable from the peripheral circulation. In like manner, the meningococcus, in cases of cerebrospinal fever, sometimes invades the blood stream. A meningococcal bacteremia is also possible without symptoms of meningitis.

Both organisms are quite fastidious in their nutritive requirement and grow poorly, or not at all, in the usual laboratory mediums; those containing albuminous material seem to be necessary for their successful cultivation. Beef heart infusion agar, or "hormone" agar to which hydrocele or ascitic fluid has been added, in the proportion of two parts of agar to one part of fluid, is a very satisfactory medium. Pour plates containing 9 c.c. of medium for each cubic centimeter of blood give the best results. In culturing meningococci in agar medium it is important that the agar be cooled to at least 40° C. before adding the blood, as meningococci are killed at 41° C. Both meningococci and gonococci are very sensitive to loss of moisture. If the water of condensation is not present, the cultures may be placed in a closed jar containing moist cotton or filter paper. Gonococci, in addition, may be cultured in beef heart infusion broth containing hydrocele or ascitic fluid in similar proportions. The optimum incubation temperature for the gram-negative cocci is about 37° C. Differentiation of meningococci and gonococci is discussed in the section on spinal fluids.

Typhoid-Colon Group.—The most favorable time for making blood cultures on patients with typhoid fever is during the first week of the disease. While the typhoid bacillus grows readily in nutrient broth, provided the P_H is from 6.8 to 7.0, the most satisfactory medium is ox bile, either alone or containing a small amount of glycerin and peptone. Test tubes containing from 7 to

10 c.c. of this medium are made up and autoclaved. It is advisable to add the blood in amounts varying from 0.5 to 3 c.c. to several tubes of bile medium. The cultures are incubated at 37° C. and subcultured every twenty-four hours on lactose litmus agar or Endo's medium. Positive results are usually obtained in seventy-two hours but may be delayed as long as five or six days. Organisms of this group are identified by their cultural characteristics, by their fermentative activities, and by agglutination tests.

It is convenient in private practice to place the bile in sterile vaccine bottles into which the patient's blood is injected. In this way specimens can be readily transported to the laboratory.

Influenza Group.—The difficulties experienced by pioneer workers in isolating organisms of this group have been lessened by more efficient methods of cultivation. Suitable mediums consist of beef heart infusion broth, or agar, containing blood subjected to tryptic or peptic digestion. Chocolate agar, prepared by adding defibrinated blood to agar medium which has been heated to 100° C. is of value.

The influenza bacilli are strictly aerobic; shallow layers must, therefore, be employed in liquid medium. If a solid medium is used, the blood should be smeared lightly over the surface. Colonies of these organisms appear as circular, colorless, translucent discs, with sharp edges and fine granulations in the center. The organisms die easily—rarely can they be kept longer than ten days. On smears the organisms appear as minute coccobacilli measuring from 0.2 to 0.8 μ, are gram-negative and stain faintly; on ordinary agar they grow as very small pinpoint colonies barely visible to the naked eye.

Brucella Group.—Members of this group may be recovered from the peripheral blood in a high percentage of cases of undulant fever. The blood is best taken at the height of an exacerbation, although organisms are occasionally recovered during afebrile periods. It may be inoculated in either liquid or solid mediums. The organisms will grow in simple nutrient broth, provided the blood is in the proportion of at least one volume of blood to eight of medium. Trypsinized broth and liver infusion broth (Stafseth's broth) have been employed with success. With liver infusion agar and glucose agar, which are sometimes used, the blood is spread directly on the surface of the plates. The most favorable temperature for incubation is 37° C. and the organisms grow best in an alkaline medium with a P_H of 7.4. Growth generally appears in from three to four days but may be delayed as long as two weeks.

The Bacillus abortus of bovine or porcine origin generally requires an environment containing a high percentage of C O_2 for growth.

The laboratory diagnosis of Brucella infections is made by isolating the organisms from the blood, by demonstrating agglutinins in the patient's serum, and by testing for skin sensitivity.

SPINAL FLUID

Collection of Specimen.—The spinal fluid may be conveniently collected in centrifuge or test tubes. Part of the material should be set aside for cell count, chemical tests, and complement fixation. The material for culture, especially when meningococci and gonococci are suspected, should be kept at body tempera-

ture by means of a water jacket and sent immediately to the laboratory for culturing. Part of the specimen may be set aside for guinea pig inoculation.

Microscopic Examination of Films.—If tuberculous meningitis is suspected, part of the fluid should be permitted to stand until a coagulum forms. This fibrin clot is then gently floated out on a slide, dried, fixed, stained according to Ziehl-Neelsen technic, and examined for tubercle bacilli. Great care must be exercised in withdrawing the clot, as touching it with a platinum loop usually causes it to form a tough mass. Examination of the pellicle is more frequently successful than examination of the centrifuged specimen.

The remainder of the spinal fluid is centrifuged for one-half hour. The supernatant fluid is poured off and smears are made of the sediment. It is well to stain slides according to the Ziehl-Neelsen technic, with Gram's stain, and with methylene blue. From the smear stained with methylene blue an estimate of the relative number of each type of leucocyte can be made.

The following organisms are differentiated by Gram's stain:

1. Gram-positive: streptococci, pneumococci, staphylococci.
2. Gram-negative: meningococci, gonococci, Pfeiffer's bacilli.

If clinical findings are taken into consideration, a tentative diagnosis can frequently be made from the examination of smears. In the case of finding tubercle bacilli, the diagnosis is definite.

As the patient's life may depend on the rapid diagnosis of meningococci in the spinal fluid, it is always well to study the smears very carefully when gram-negative cocci are found. The textbook picture of stained smears of the cerebrospinal fluid showing large numbers of kidney-shaped cocci arranged in pairs with the indented surfaces opposed to one another is seldom seen in the clinical laboratory. There is a great diversity in the microscopic appearance of films of the spinal fluid from patients with meningococcal meningitis. The cocci may be few or numerous; largely intracellular or extracellular. They may be in pairs or tetrads, but often are found singly. It is interesting to note that when meningococci are found singly they are usually round or elliptical, seldom giving the typical flat kidney-shape picture. When rapid diagnosis is requested, a positive report for meningococci may be made whenever gram-negative cocci are found in the spinal fluid. The administration of antimeningococcic serum is justified even when organisms are not found in the smears, provided the leucocyte cell count is high and the smear demonstrates that this increase is largely due to polymorphonuclear cells. Of course, it is advisable in such instances to take into consideration the patient's clinical picture.

Cultural Examination.—The diagnosis of tubercular involvement of the meninges is frequently made from careful examination of smears only; however, if tuberculosis is suspected and direct microscopic examination has failed to demonstrate organisms, cultures and guinea pig inoculation should be carried out. Cultures of the fluid, especially of the pellicle, on Petroff's medium is the method of choice.

The gram-positive cocci, streptococci, pneumococci, and staphylococci grow readily in blood agar plates and beef heart infusion blood broth. In liquid media the addition of 1 per cent dextrose or the substitution of 0.2 per cent sodium

phosphate for the sodium chloride content is sometimes used to enhance growth. The optimum P_H and temperature for incubation are 7.6 and 37° C., respectively. The organisms are differentiated by their characteristic growth in blood broth, and blood agar and, further, by the examination of smears. The bile solubility test is frequently necessary to distinguish pneumococci from streptococci.

Of the gram-negative cocci the meningococcus is by far the most important, and is found with some frequency while the gonococcus is recovered only rarely from the spinal fluid. They may be appropriately considered together, as they both require a highly albuminous medium for growth. A blood agar plate containing from 30 to 50 per cent hydrocele or ascitic fluid is the medium of choice. It is necessary that the medium be freshly prepared and that the specimen be cultured as soon as possible, as these organisms are markedly susceptible to cold or drying. Inoculation of the medium is best carried out by heavily streaking the plates with the spinal fluid, and in addition, pour plates may be made with this medium. The same precautions for possible loss of moisture should be observed as indicated in the section on blood cultures. Another useful method for culturing meningococci is to incubate the fluid for an hour or two before streaking plates.

Colonies of meningococci on clear mediums are transparent, lenticular in shape, and have a moist and smooth surface. Meningococci cannot be definitely differentiated from gonococci by manner of growth in culture mediums or by smear findings, nor does the examination of their fermentative activities solve the question. The ultimate diagnosis of meningococci must, therefore, depend on the agglutination reactions. A polyvalent immune serum is generally used for this purpose but when a fine differentiation of meningococcal types is desired, it is necessary to perform these tests with the three types of immune serum.

DISCUSSION

No attempt has been made to discuss every form of microorganism met with in blood or spinal fluid cultures; only those frequently found, or of practical importance are included.

With the realization that the tentative clinical diagnosis is frequently wrong, it is always advisable to perform routine examinations, as well as to carry out any special studies indicated.

Too much emphasis should not be placed on negative results. Many bacteremias have bacteria-free stages. In most cases this period is short, but in such diseases as subacute bacterial endocarditis it may cover a period of months.

The clinical interpretation of laboratory findings is sometimes very important. For instance, streptococci have been isolated from the blood of patients with measles, chronic arthritis, rheumatic fever, and even upper respiratory infections. To conclude that such patients have a streptococcal septicemia in the usual sense of the term would be erroneous. Such organisms are usually isolated by special technic, and are probably of low virulence; they may be present in small numbers, and possibly at infrequent intervals. It is also conceivable that such organisms are present in the blood stream only in some ultramicroscopic form, later developing into the visible stage of their life cycle in

the blood culture medium. The question of secondary or terminal invaders also has to be considered.

In the average laboratory, unusual or unlooked-for organisms are generally discarded as contaminants. It is here recommended that such organisms be given careful consideration, as it is largely through study of the unexpected that our bacteriologic knowledge is increased.

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THE BACTERIOLOGY OF THE NOSE AND THROAT*

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IF WE consider the nose and throat as vestibules to the respiratory and alimentary tracts, and the relations of their vascular and lymphatic systems to the general circulation and the meninges, we are impressed by the avenues afforded to pathogenic bacteria for invasion and absorption of their toxic products. Further, a consideration of the diseases which may be spread in droplets of nasal and throat secretions emphasizes the importance of the clinical bacteriology of this region.

For convenience we shall discuss separately the flora of the nasal passages, the accessory nasal sinuses, the nasopharynx, and the tonsils; but such a division is very inexact bacteriologically, because the continuity of the different parts, the constrictions and tortuosities of the passages, and the admixture of their secretions make it impossible to secure material from any one limited area uncontaminated by the bacteria from another.

Material for examination may be obtained by rubbing a sterile swab over the mucous membrane or by irrigating the passages with warm sterile salt solution, Ringer's solution, or broth. The use of a swab is a most convenient method and the only practical one when the secretion from a limited area is desired. To take material from the nasopharynx, a bent swab may be inserted through the mouth and behind the soft palate and rubbed over the nasopharyngeal walls. With proper care on insertion and withdrawal, there is little danger of contamination from the mouth or tongue. In young children, or adults with exaggerated

*From the Bacteriological Laboratory of the Medical Division, Metropolitan Life Insurance Company.

gag reflexes, the West tube may help to minimize contamination of the swab with saliva, or a slightly bent swab may be passed through the middle meatus of the nose into the nasopharynx. Material taken in the latter way is necessarily contaminated by bacteria from the nasal passages. Irrigation of the nose and nasopharynx with a sterile solution may be employed when large amounts of secretion from the whole region rather than from one locality are desired. It is the method used by Olitsky and Gates,[1] and other workers in studying the etiology of colds and influenza, and is especially useful when filter-passing forms are under investigation. Dochez[2] suggested sterile broth as the irrigating medium, because he believed that it preserved these bacteria and favored their filtration. Such material is frequently contaminated by bacteria from the mouth and nasal vestibule, and in comparison with nasopharyngeal swabbings contains fewer pneumococci and influenza bacilli, and more staphylococci of the albus variety, and diphtheroids.

Direct microscopic examination of the secretions is of limited value unless there is a demonstrable lesion with an exudate. Cultivation on suitable media will frequently show the presence of a suspected pathogen when direct microscopic examination fails to do so. Obviously the media employed must be adapted to the growth requirements of the particular organisms searched for, as for example, Loeffler's coagulated serum medium for the diphtheria bacillus, or hemoglobin media for the hemoglobinophilic group.

In discussing the bacteriology of any region an attempt is usually made to distinguish "normal" from "abnormal" flora. The reasons are obvious, but an exact differentiation is not possible because the flora is never constant; it varies in different persons, in the same person at different times, and in different geographical localities.[3, 4, 5] Broadly speaking, certain species of bacteria occur more or less regularly in most healthy noses and throats, and may be considered "normal" flora. They are for the most part saprophytes and potential pathogens of low virulence. It is doubtful if virulent pathogens are ever normal flora, unless they be considered such in carriers in whom there are no demonstrable foci of infection. The "abnormal" flora consists of bacterial species occurring less constantly than those of the normal flora; it is transient except in persons with chronic lesions or foci of infection, and includes the more virulent pathogenic forms.

THE NOSE

The bacterial species of the healthy nasal mucosa have been listed by Bloomfield,[6] Shibley, Hanger and Dochez,[7] and Noble, Fisher and Brainard.[4] Bloomfield considered that Staphylococcus albus and diphtheroids comprised the essential normal flora of the nose, and that gram-negative cocci, B. lactis aerogenes and hemolytic streptococci were normal transient forms. Dochez and his associates[7] considered that staphylococci of the aureus and citreus varieties also belonged to the normal flora, and Noble, Fisher and Brainard[4] found that green streptococci and pneumococci were occasional transients. Webster and Hughes,[8] in studying the incidence and spread of pneumococci in healthy persons, found these organisms more frequently in the nasal passages of children than in their

throats, but in adults they found them less frequently in the nose. We may therefore conclude that the normal flora of the nose consists principally of diphtheroids and staphylococci of different varieties, that gram-negative cocci (M. catarrhalis, M. crassus, M. flavus and M. siccus) and pneumococci are common transients, and that green and hemolytic streptococci occur but rarely as normal transients.

The abnormal flora of the nose is complex, and its clinical significance is by no means clear, especially in the acute catarrhal diseases. Mackey[9] reported finding B. influenzae, B. pertussis, and B. mucosus capsulatus in cases of chronic nasal catarrh in children. Bordet[10] and Monerieff and Lightwood[11] have found B. pertussis in the nasal mucus and muco-pus of children with whooping cough. Meyer and Steinert[12] isolated at autopsy the bacillus of Koch-Weeks from the purulent secretion of the nasal passages and from the spinal fluid of a fatal case of meningitis. Schulman[13] and Hollender[14] have reported nasal infections caused by Vincent's fusiform bacilli and spirochetes. Kistner[15] found tubercle bacilli in primary tuberculosis of the nasal mucosa. Gonorrheal rhinitis with isolation of the gonococcus from the nasal pus was reported by Miller.[16] Several varieties of gram-negative encapsulated bacilli (B. mucosus capsulatus group) have been found associated with rhinoscleroma and ozena. Jelin[17] believed that they had no causative relationship because so many different fermentative varieties occurred in the same patient. Elbert and Guerkess,[18] however, considered one type, the bacillus of Frisch, characterized by the formation of acid from glucose and saccharose only, to be of significance because of its presence within the infiltrations of granulomatous tissue together with the formation of specific immune bodies in the blood stream of patients. In cases of glanders, B. mallei is to be found in the nasal discharge by suitable methods of examination. The Klebs-Loeffler bacilli may be demonstrated in cultures from the nose in cases of nasal diphtheria.

THE ACCESSORY NASAL SINUSES

Because of the difficulty in securing uncontaminated material from the healthy sinuses in the living subject, our most accurate information of their bacteria has come from studies made upon the sinuses of cadavers. Fraenkel[19] examined 50 sinuses at autopsy; 28 were apparently healthy, and cultures from 13 of these showed no growth. In the remaining 22 sinuses (11 maxillary, 6 frontal, and 5 sphenoidal), pneumococci were the predominating bacteria found. Linton[20] studied normal sinuses in 26 cadavers within five to eighteen and one-half hours after death. After removal of the calvarium and brain, the inner surface of the bone overlying the sinuses was sterilized by heat and trephined. Only the frontal, sphenoidal and ethmoidal sinuses could be reached in this way; 74 per cent of the sinuses were sterile; the remaining 26 per cent yielded cultures of staphylococci, diphtheroids, B. coli, hemolytic streptococci, M. catarrhalis, and sarcinae. In examining a large series of maxillary sinuses in animals, he found that 55 per cent in rabbits, 71 per cent in dogs, 83 per cent in guinea pigs, and 90 per cent in rats were sterile. It would therefore seem justifiable to conclude that the majority of healthy sinuses are bacteria free, and that if bacteria gain entrance they are probably transients.

A number of different bacterial species has been found associated with inflammatory conditions of the sinuses. In 44 cases of acute sinusitis Babcock[21] reported finding pure cultures of pneumococci in 24, staphylococci in 10, streptococci in 2, M. catarrhalis in 2, diphtheroids and B. aerogenes in 1 each, and no organisms in 4. In chronic cases the same author found staphylococci in pure culture 15 times, streptococci 4 times, pneumococci 3 times, and B. mucosus capsulatus, M. tetragenous and diphtheroids, once each. Ashley and Frick[22] found in the maxillary sinuses of children Staphylococcus aureus in 23, M. catarrhalis in 18, Staphylococcus albus and green streptococci in 10 each, and B. influenzae, B. pyocyaneus, diphtheroids, and gram-negative encapsulated bacilli in one or two cases each. Ersner[23] in analyzing 50 operative cases of maxillary sinusitis reported that hemolytic and green streptococci predominated, but were more often found in nonsuppurative conditions. Tubercle bacilli[24, 25] and Vincent's fusiform bacilli and spirochetes (anaerobic forms)[26] have been reported as causative agents in maxillary sinusitis. B. mallei has also been found in infections, especially of the maxillary and frontal sinuses.

THE NASOPHARYNX

The bacteria commonly found in the healthy nasopharynx have been reported upon by Jordan, Norton and Sharp,[27] Noble, Fisher and Brainard,[4] Burky and Smillie,[5] and Milam and Smillie.[28] Their results are based upon successive cultures taken from the same persons over long periods of time or upon single cultures from larger groups of subjects, the first method being of value in showing transient flora and the length of time it persists. There appears to be general agreement that green streptococci and gram-negative cocci are the predominating normal or basic flora of the healthy throat; that indifferent streptococci (Brown's "gamma" type),[29] pneumococci, staphylococci, nonhemolytic influenza bacilli, and diphtheroids are normal flora in some persons and frequent transients in others; and that hemolytic streptococci, M. catarrhalis and B. mucosus capsulatus are occasional transients. Burky and Smillie,[5] and Milam and Smillie[28] found the normal basic nasopharyngeal flora in isolated communities in the subarctic, temperate and tropical zones to be, in general, the same with some minor differences: pneumococci were not found in normal throats in Alabama while they were quite prevalent in Labrador and the Virgin Islands; influenza bacilli occurred infrequently in Labrador; hemolytic staphylococci were very common in the Virgin Islands.

The variations in the nasopharyngeal flora in health make difficult a determination of the etiologic agents in acute catarrhal infections of the upper respiratory tract. There are already recorded in the literature countless attempts to determine the significance of certain organisms of this region in colds and in influenza. Pfeiffer's bacillus, discovered in 1892, was generally believed to be the cause of epidemic influenza until the pandemic of 1917-1918. During that outbreak Williams, Nevin, and Gurley[30] found it in 92 per cent of influenza cases and in only 40 per cent of healthy persons, but attempts to demonstrate a common epidemic strain resulted in showing such serologic heterogeneity as to cast doubt upon its etiologic significance. Mathers[31] described a hemolytic

streptococcus found in the nose and pharynx of influenza patients during an outbreak in Chicago in 1915-1916. Attempts to implicate green streptococci have been made by numerous workers. In colds, changes in the normal flora have been noted by different investigators. Williams, Nevin, and Gurley[30] found that pneumococci, staphylococci and influenza bacilli were increased in number and frequency. Shibley, Hanger, and Dochez[7] found that staphylococci of the aureus variety, hemolytic streptococci and influenza bacilli appeared late in colds, probably as secondary invaders. Noble, Fisher, and Brainard[4] observed that pneumococci, staphylococci, indifferent streptococci, and influenza bacilli were increased during colds. Burky and Smillie[5] noted in Alabama the appearance of pneumococci of Group IV together with an increase of influenza bacilli when colds developed, while in Labrador an increase in the prevalence of influenza bacilli was associated with an epidemic of tracheitis.

Tunnicliff and Hoyne,[32] while studying measles, isolated a green diplococcus which at first grew only anaerobically, but in later generations developed under aerobic conditions. They reported that serum from goats immunized to this organism seemed to afford some protection to persons who received it within three days of exposure.

The significance of certain organisms in the nasopharynx is well understood, as for example, the Klebs-Loeffler bacillus in diphtheria, and the hemolytic streptococci in scarlet fever and in septic sore throat. The presence of these virulent organisms in the throats of healthy persons would indicate that the latter are carriers. Meningococci may be found in the nasopharynx during the disease and also in carriers. B. mallei has been demonstrated in the pus from the nasopharynx in glanders, and the Spirocheta pallida in the mucous patches of secondary syphilis. Tubercle bacilli have been found in tuberculous ulcerations of the nasopharyngeal mucosa.

THE TONSILS

The flora of the normal tonsil has been reported by Bloomfield[6] and others to consist of gram-negative cocci, Streptococcus viridans and diphtheroids, with Staphylococcus albus, Staphylococcus aureus, hemolytic streptococci and hemolytic influenza bacilli occurring as transients. Cobe[33] has found in addition pneumococci and B. mucosus capsulatus.

Many of these groups undoubtedly contain potential pathogens, the presence of virulent strains among them remaining undetected until an outbreak of disease. The Dicks have demonstrated that scarlet fever is produced by hemolytic streptococci of a specific type. Bloomfield and Felty[34] found that certain persons consistently harbored hemolytic streptococci in their tonsils and others did not. Acute tonsillitis with the coincident appearance of hemolytic streptococci developed among the second group while the first remained healthy. They therefore believed that hemolytic streptococci were the inciting agents, and attributed the immunity of those in the first group to the fact that they were carriers. In a study of excised tonsils from 100 cases of acute tonsillitis, Polvogt and Crowe[35] found hemolytic streptococci the predominating organisms in 91 per cent. Pomales[36] in his examination of 65 pairs of excised tonsils found

Staphylococcus aureus the predominating form in the majority of cases, with hemolytic streptococci ranking second, and considered both organisms to be of significance.

The possible relationship to rheumatic fever of certain strains of streptococci of the throat has been suggested many times; it presupposes the existence of one or more specific types and their recovery with regularity from the throats, blood, and affected joints of rheumatic fever patients. These conditions have not yet been satisfactorily fulfilled. A mass of conflicting evidence has accumulated which is difficult to appraise. Andrewes, Derick, and Swift[37] found no evidences of serologic identity among their strains of hemolytic streptococci isolated from the tonsils, throats or hearts' blood of rheumatic fever patients. Small[38] and Birkhaug[39] have recently described stains of indifferent streptococci (gamma type) isolated from the throats, blood, urine, or feces in acute rheumatic fever, and each has stated that his strains constituted a serologically homogeneous group. Nye and Seegal[40] were unable to confirm these observations, and Hitchcock[41, 42] has found the indifferent streptococci to be heterogeneous, and the different strains to occur with equal frequency in the throats of rheumatic fever patients and normal individuals. The work of Cecil, Nicholls, and Stainsby[43] is of especial interest, for they have isolated green streptococci in a high percentage of their cases from the blood and affected joints, and have proved the serologic identity of strains recovered from the two sources.

If the tonsils are involved in diphtheria, cultures from them will show diphtheria bacilli. Other bacteria which may produce diphtheria-like conditions have been reported. Gilbert and Stewart[44, 45] have recently described a pathogenic diphtheroid associated with outbreaks of sore throat; young cultures showed diphtheroid forms which became coccoid on longer incubation. Diphtheria antitoxin afforded little protection. Two cases of tuberculosis of the tonsils have been reported by Koplik[46] and Dickey.[47] In both, microscopic examinations of scrapings from the ulcers showed acid-fast bacilli. The case described by Dickey was probably a primary infection, as a tuberculin test made one and one-half years before the appearance of the ulcer was negative, while one made afterwards was positive. Foster[48] reported acute tonsillitis in a patient who had contracted gonorrhea; a microscopic examination of smears from the tonsillar ulcer showed intra and extracellular gram-negative cocci, and organisms morphologically and culturally resembling the gonococcus were isolated. Busson[49] found B. mucosus capsulatus (Friedlaender's bacillus) in pure culture in a peritonsillar abscess. B. mallei may be found in the discharge from the nodules developing on the tonsils in glanders.

Diseases of the tonsils produced by fungi have been described by Castellani.[50] In follicular tonsillomycosis and diphtheria-like tonsillomycosis the conditions were usually caused by yeast-like fungi of the genus Monilia but occasionally by other types. Hoffstadt,[51] from a white tonsillar membrane, isolated a saccharomyces which resembled that found by Kayser[52] in cider. Smith[53] reported a case showing whitish patches on the tonsil, base of tongue, and lingual tonsil; an examination of the excised tonsil showed the presence of actinomyces. Rarer infections with the formation of granules in the tonsillar crypts have

been reported by Castellani.[50] The granules contained masses of nocardia-like organisms, leptothrix and vibriothrix, various bacteria and even protozoa such as spirochetes, amebae, and flagellates. Tunnicliff and Jackson[54] reported finding a vibriothrix in a tonsillar granule. Petzetakis[55] found amebae in the tonsils of two children with sore throats a few days before they developed amebic dysentery.

ANAEROBIC BACTERIA

The demonstration of anaerobic bacteria in the upper respiratory tract is complicated by the ability of most of the aerobic forms previously named to grow well under anaerobic conditions. A partial solution of this difficulty lies in filtering the respiratory secretions through Berkefeld "V" or "N" candles before planting them in culture media. Most of the aerobic bacteria and some of the anaerobic forms are removed by this procedure, but many of the latter pass through with the filtrate.

Some anaerobic bacteria, however, have been isolated from unfiltered material from the nose or nasopharynx. Tunnicliff's[56] B. rhinitis was found in the unfiltered nasal mucus of patients with acute coryza; she believed it to induce this condition when inoculated upon the nasal mucosa. Noble and Brainard[57] found Staphylococcus parvulus in unfiltered nasal washings. This organism, previously isolated by Veillon and Zuber[58] from an infected appendix, and later by Lewkowicz[59] and others from the mouths of man and animals, is characterized by its ability to produce abundant gas in protein media and its inability to ferment sugars. Because of its prevalence it would seem to be part of the normal nasopharyngeal flora. Several strains of gram-positive cocci, differing from one another in their fermentative reactions have been isolated from cases of rhinitis by Hall,[60] and Noble and Brainard.[57] Gram-positive spirochetes have been found by Tunnicliff[61] in cases of rhinitis and sinusitis. Gram-negative bacilli similar to those found in filtered washings, which are described below, have been reported by Noble and Brainard[57] in nasopharyngeal swabbings from normal persons and others with colds. Vincent's fusiform bacilli have already been mentioned in an earlier section.

The filter-passing anaerobic bacteria which have been described at one time or another are more numerous than those obtained directly from the unfiltered mucus. Olitsky and Gates[62] isolated a gram-negative bacillus from filtered nasal washings of patients in the early stages of epidemic influenza and suggested it as the causative agent; they called it B. pneumosintes because of its ability to injure the lung and predispose to secondary infections. When grown in Smith-Noguchi medium, it is a minute coccobacillus; on a solid medium such as coagulated blood agar, it is considerably larger. It produces acid from glucose, galactose, levulose, maltose, saccharose and lactose, and specific agglutinins and other immune bodies in human beings and rabbits. Many other gram-negative bacilli have been found by different workers. Olitsky and Gates[63] described three groups and two subgroups, and Noble and Brainard[64] have differentiated twelve agglutinative and fermentative groups; these have been isolated from cases of epidemic influenza, rhinitis, or healthy subjects, and are probably part of the normal flora of the upper respiratory tract. Garrod[65] has reported a minute

gram-negative coccus occurring in hazy masses, and Noble and Brainard[64] have found a similar form. It produces a characteristic colony on glucose serum agar but does not ferment the sugars. It is not pathogenic and is found in the majority of persons. Two species of gram-negative cocci have been described by Branham,[66] one of which appeared to be Staphylococcus parvulus, while the other differed from it in that it was hemolytic and formed no gas in protein media. Branham also found a minute gram-positive coccus. We have found small gram-positive bacilli, some resembling diphtheroids.

SUMMARY

From the foregoing paragraphs, it is evident that many bacterial species are to be found in the nasal passages and throat, and that the same, or apparently the same species occur on both the healthy and diseased mucous membrane. Therefore it is difficult, in the absence of a definite lesion, to appraise satisfactorily the clinical significance of many of the species encountered in a bacteriologic examination. This is especially true of the streptococci, whether green, indifferent or hemolytic, the pneumococci, staphylococci, gram-negative cocci and hemoglobinophilic bacilli. If we consider the hemolytic streptococci as an example, we must remember that they comprise a large group of many strains which differ greatly in their pathogenicity and virulence, and that while certain strains may give rise to scarlet fever, or to septic sore throat, others may be innocuous. The finding of hemolytic streptococci, therefore, is not necessarily significant, but the finding of a particular strain may be of great significance. Unfortunately the demonstration of a particular pathogenic strain is not always easy and is usually impractical in routine examinations. The rôle of the anaerobic bacteria as incitants of colds and influenza is unknown; they would appear to be part of the normal flora and of little if any pathogenicity, but additional experimental evidence may change our views. The recent work of Dochez,[67] confirming the earlier work of Kruse[68] and Foster,[69, 70] has again directed our attention to the probability that a filterable virus is the primary cause of certain respiratory infections, preparing the way for the secondary complications which may be brought about by many of the common bacteria harbored in the upper respiratory tract. We are probably correct in believing that in the presence of a definite lesion the finding of an organism of known or potential pathogenicity is clinically significant; that in the absence of a lesion it denotes a carrier, but beyond this our present lack of knowledge does not justify definite interpretations.

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THE BACTERIOLOGY OF BILE

Obtained by Duodenal Tube Biliary Drainage

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THIS article deals with the bacteriologic study of 1,450 cultures made from the bile of 988 patients obtained during the course of duodenal tube drainage of the biliary system, following the technic which we have advocated. That is, bile was allowed to flow from the patient through the duodenal tube into a culture flask containing Huntoon's hormone broth, 20 to 30 drops of bile fluid being introduced into 100 c.c. of broth. In this sense, therefore, the title of this article is correct. But from another point of view all findings accredited to the bacteriology of the bile are justifiably debatable.

In the first place in taking cultures through a duodenal tube from the duodenum we are obviously culturing a zone into which is being poured secretions from sources other than the biliary system, namely, the secretions of the respiratory tract, of the mouth (notably the saliva), of the stomach, the pancreas, and the duodenum itself. Viable bacteria from any of these sources ultimately reach the duodenum if they can successfully pass such bactericidal barriers as our bodies possess. Obviously, this would make inferences as to the primary source of positive cultures from the duodenal zone somewhat doubtful. Scientifically speaking this is the sounder view to take and this position has emphatically been chosen by some investigators of this problem. However, I believe and hope to show that in many patients, despite certain obvious inaccuracies, bacteriologic inferences can be made which are of such value both in diagnosis and in treatment as to make it imperative that we do not entirely disregard this aid in diagnosis.

In medicine, as in other branches of science, the ultimate truth is long delayed. The opinions expressed in one decade, or one medical generation, or in one century are often fully accepted for the time being, and often totally disregarded by the next generation because methods have been improved for reappraising former views.

Thirty years or more ago it was generally accepted that the *fasting duodenum in health* was amicrobic; whereas bacteria ingested with the food and contaminated by bacteria resident in the mouth and upper and lower respiratory tracts, resistant to the socalled bactericidal power of the acid gastric juice, could be readily cultivated from the duodenum when operated upon. Such cultures chiefly yielded yeast and various cocci.[1] Twenty years ago, after the duodenal tube had been perfected, other investigators[2, 3] showed that viable bacteria could be readily cultivated from the fasting duodenum in health and also with particular frequency in gastrointestinal disease. However, the view was held that such findings were inaccurate because of contamination by

bacteria carried from the mouth, esophagus, and stomach by the duodenal tube itself. Thirteen years ago, after the advent of nonsurgical drainage of the gall tract,[4] we added routine cultures from the duodenal zone and became enthusiastic in our belief that, with certain precautionary attempts to sterilize the mouth and stomach, cultures could be secured which would furnish inferential evidence of the source from which such bacteria were obtained. Following my earlier publications[5] renewed interest was indicated in various papers.[6-20] The opinions of some of these authors supported, while others were unable to confirm the views which I had expressed, chiefly because of the obvious factors of contamination. No one can dispute or disregard the existence of such factors. It is necessary, however, to emphasize that with care in technic such factors can be at least partially controlled.[5-k]

In health the mouth, its contents, and the accessory cavities which empty into it (the respiratory passages, sinuses, and ears) constantly contain various bacteria, many of which are pathogenic, but the normal resistance of the tissues and the antibacterial defenses (lymph nodes, etc.) minimize their effect. In disease (bronchitis, nasopharyngitis, sinusitis, otitis media, eustachian-tube infection, gingivitis, pyorrhea, dental caries and root infection, tonsillitis, salivary duct infection) the mouth cavity is a most potent source of infection through lymphatic and blood distribution and by direct bacterial descent (swallowed saliva) in the production of subsequent gastrointestinal disease in its acute and chronic forms (gastroduodenitis, peptic ulcer, cholecystitis, hepatocholangitis, cirrhosis, enteritis, appendicitis, colitis). In the preceding groups the pyogenic cocci are particularly common. One of the greatest factors influencing the causation of such secondary disease is the individual tissue susceptibility and the tissue resistance to such infection. A second factor of less importance is the bactericidal power of the acid gastric juice and its various components.[21]

Every gastroenterologist is at times amazed to see the frequency of extensive oral sepsis, especially in patients from rural communities, which exists in certain patients without evident acute or chronic gastrointestinal disease or of infectious arthritis, endocarditis, neuritis, and the like. No doubt, many such persons acquire slowly developing immunity to the bacteria harbored in such dirty mouths. On the other hand, even in health various air-borne bacteria, such as influenza, may overwhelm the person and cause acute intestinal disease; likewise various food, water, or insect-borne bacteria may produce acute endemic or epidemic enteric disease by overwhelming the nonimmune host, as occurs in typhoid, paratyphoid, and various other bacillary and coccus infections. It would seem, therefore, that much depends on the virulency of the bacterium measured in terms of tissue resistance and in the selective affinity of certain tissues to certain strains of virulent bacteria, as pointed out by Rosenow.

The 988 cases on which this study is based occurred in my private practice between the years of 1920 and 1926. These patients were going through the usual routine diagnostic appraisal for gastrointestinal disease or its absence. All of them had one or more cultures made of bile discharged into the duodenum and recovered by the duodenal tube. Of these 988 patients 554 were males and 434 were females. Classified by half decades from youth to old age, Table I indicates that over two-thirds of the patients were between the ages of thirty and

fifty-four years, and the largest number of patients for any single decade was between thirty-five and forty-four years.

Of the total number of patients, 64 furnished no evidence of gastrointestinal disease after complete study and represent the average normal adult. This group furnished the highest percentage of sterile cultures. A second group of 93 patients on final appraisal represented cases of various functional gastrointestinal disorders. This group furnished the next highest percentage of sterile cultures. A third group of 162 cases on final appraisal represented various grades of organic gastrointestinal disease, but without recognizable clinical or laboratory involvement of the biliary system. Of these, the 32 who came to the operating table showed no evidence of gall bladder disease. A fourth group of 404 patients were cases of biliary tract disease, easily recognized by history, physical examination, roentgen ray examination, laboratory studies, including biliary drainage, and in 101 instances were confirmed by primary gall bladder surgery. I consider it significant that in these 101 patients the culture obtained at the operating table from the gall bladder was identical with that recorded in the preoperative drainage culture in a definite majority of cases. A fifth group of 69 cases represented patients who had already undergone one or more operations on the biliary system (cholecystostomy, cholecystectomy, cholecystoduodenostomy, release of adhesions, etc.) and who suffered continued morbidity. A sixth group of 154 patients presented vague atypical symptoms that were not characteristic of

TABLE I

988 PATIENTS ANALYZED BY AGE AND SEX

Years	10 to 14	15 to 19	20 to 24	25 to 29	30 to 34	35 to 39	40 to 44	45 to 49	50 to 54	55 to 59	60 to 64	65 to 69	70 to 74	75 to 79	Total
Males	3	6	22	46	76	86	88	68	66	36	29	24	2	2	554
Females	3	4	22	49	54	83	66	44	54	24	12	17	2	0	434
Total	6	10	44	95	130	169	154	112	120	60	41	41	4	2	988

any of the usual gastrointestinal or biliary tract syndromes, and who would otherwise have remained unrecognized as biliary tract suspects or Grades I or II biliary tract disease, were it not for the evidence of abnormal cytology and positive cultures of pathogenic bacteria obtained by biliary tract drainage. This group, most important to recognize at an early stage of their disease, yielded 15 per cent of the positive cultures. This group also yielded the largest number of successful results from therapeutic biliary drainage by duodenal tube. A seventh group of 74 patients were cases which clearly overlapped, chiefly with Groups 4 and 5, and represented various types of liver disease (cirrhosis, hepatitis, cholangitis, cancer).

The histories of this series of 988 cases showed that only 333 patients had not been operated upon for any condition. Of the 988 patients 316 had already undergone 434 abdominal operations. In many instances two or more abdominal organs had been surgically treated at the one operation, so the following figures overlap: 269 patients had already had their appendices removed; 69 patients had previously undergone gall bladder surgery, of which 34 were cholecystos-

tomies, 32 cholecystectomies, and 3 cholecystoduodenostomies. Of this group I had to refer 14 patients for further gall tract surgery. The remainder of this group of 316 cases were made up of pelvic surgery, gastrojejunostomy or other operations for peptic ulcer, colostomy, release of adhesions, and hernial repairs. Of this group having had abdominal surgery 228 had been operated on once, 63 patients twice, 20 patients three times, and 5 patients four times. From the foregoing it will be noted that I had to refer 147 patients for abdominal surgery —101 for primary gall bladder operations, 14 for reoperation on the gall tract, and 32 for surgery other than gall tract.

Although this data may appear irrelevant to the subject of this paper I feel that it is necessary to tabulate and classify the general character of the material studied by duodenobiliary culture, because it serves better to account for bacteriologic discrepancies between this series and others that might be studied in a more general practice.

In addition, there were among the 988 cases 339 patients who had been operated for various conditions requiring nonabdominal surgery (tonsils, adenoids, turbinates, sinuses, hemorrhoids, pelvic plastic repairs, etc.). Of this group 210 patients had been operated upon once, 93 patients twice, 31 patients three times, 10 patients four times, and 4 patients five times. Many of these patients had also undergone one or more abdominal operations. For instance, one patient had had four abdominal operations and five otherwise. Two hundred and seventy-four patients had already had their tonsils removed, in whom this source of contaminative infection in the cultures reported was eliminated; and I recommended tonsillectomies in 156 patients who had evident tonsillar focal infection.

Although, in the preparation of this paper insufficient time was available to classify the number of patients who had gingivitis, pyorrhea, dental caries or root infection, previous histories of or present symptoms of sinusitis, a cursory study of the case records suggested at least 25 per cent of such present or antecedent involvement. It is evident then how important such focal infection above the level of the stomach can be in the etiology of gastrointestinal disease.

TABLE II

SUMMARY OF BILE CULTURES

	PATIENTS	CULTURES	STERILE	CONTAMINATED	PURE	MIXED
Total No.	988	1450	240	188	629	393
Per cent			16.5	12.9	43.4	27.1

An analysis of antecedent or present skin jaundice in the total group of 988 patients was significant in that of 274 patients who gave such histories, 61.8 per cent yielded positive cultures from the duodenobiliary fluid. It was interesting to me that of a total group of 184 patients who gave histories of an antecedent typhoid fever, only 3 patients yielded bile cultures of B. typhosus.*

*One of these cases was reported on page 522 of the writer's monograph (see Ref. 5-K); a second one of a typhoid carrier of twenty-six years' duration, finally cured, will shortly appear in the *Journal of the American Medical Association.*

Table II indicates that on the 988 patients a total of 1,450 cultures of the duodenobiliary fluid were made. Of these, 240, or 16.5 per cent, yielded sterile cultures; 629, or 43.4 per cent, yielded pure cultures of one bacterium only; 393, or 27.1 per cent, yielded mixed cultures of two or more bacteria; 188, or 12.9 per cent, yielded contaminated cultures.

We considered the cultures contaminated when they yielded B. subtilis, yeast, leptothrix, mycelia, or other moulds.

B. subtilis occurred 28 times or 14.9 per cent of the contaminated group, but only 1.9 per cent of the total number of 1,450 cultures; yeast occurred 16 times or 8.4 per cent of the contaminated group but only 1.1 per cent of the total number of 1,450 cultures; moulds occurred 12 times or 6.4 per cent of the contaminated group but only 0.8 per cent of the total number of 1,450 cultures. We also considered as contaminants Streptococcus salivarus, Staphylococcus albus, the diphtheroids, Micrococcus catarrhalis, and the pneumococcus.

Streptococcus salivarus occurred 19 times or 10.1 per cent of the contaminated group but only 1.3 per cent of the total number of 1,450 cultures; Staphylococcus albus occurred 71 times, or 37.7 per cent of the contaminated group but only 4.9 per cent of the total number of 1,450 cultures; diphtheroids occurred 15 times or 7.9 per cent of the contaminated group but only 1.03 per cent of the total number of 1,450 cultures; Micrococcus catarrhalis occurred 16 times or 8.4 per cent of the contaminated group but only 1.1 per cent of the total number of 1,450 cultures; pneumococcus occurred 11 times or 5.8 per cent of the contaminated group but only 0.8 per cent of the total number of 1,450 cultures. In many instances with B. subtilis, yeast, or moulds, freshly examined spreads of bile-stained floccules indicated that other bacillary or coccoid groups were present but failed to grow out in differential subcultures.

Table III indicates that in the pure cultures, in which only one bacterium was isolated, the three main groups were the streptococci, 25 per cent; the Staphylococcus aureus, 34.6 per cent; and the B. coli group, 39.3 per cent, which together represent 98.9 per cent of the total. The remainder are divided between B. pyocyaneus, B. lactis aerogenes, B. typhosus.

TABLE III

SUMMARY OF PURE CULTURES

CASES	STREPTOCOCCUS				STAPH. AUREUS	B. COLI	B. PYOCYAN.	B. LACTIS AEROG.	B. TYPHOSUS
	HEMOL.	NONHEMOL.	VIRID.	TOTAL					
Total No. (629)	93	59	5	157	218	247	3	1	3
Per cent	14.8	9.4	0.8	25.	34.6	39.3	0.47	0.15	0.47

Of the 393 mixed cultures containing two or more bacteria the four main groups are: B. coli and Staphylococcus aureus; B. coli and nonhemolytic streptococcus; B. coli and Staphylococcus albus; nonhemolytic streptococcus and Staphylococcus aureus. In these groups B. coli occurred 194 times. If these were added to the 39.4 per cent of pure B. coli cultures, B. coli would dominate

the incidence of all other bacteria. This may be important in estimating the influence of B. coli in contributing to the production of the symptoms of hepatic intestinal toxemia. It might be even higher than this percentage if the formula of Huntoon's hormone broth were not titered to retard the growth of B. coli.

While giving due consideration to the fact that the pyogenic cocci (streptococci 25 per cent and Staphylococcus aureus 34.6 per cent) which occurred in 59.6 per cent of all pure cultures, might also represent contaminations from above the duodenal zone in many instances, perhaps even in a majority, nevertheless, an analysis of Groups 3, 4, 5, and 7 will indicate that in many patients these bacteria also invade the biliary tract and produce localized infection which can best be detected, prior to operation, by cultures of the duodenobiliary fluid obtained by the duodenal tube.

Group 3 (Table IV) consisted of 162 cases of organic gastrointestinal disease (chiefly gastroduodenitis, stomach or duodenal ulcers, upper right quadrant adhesions, appendicitis, colitis), but without recognizable biliary tract disease. Of these, 42 were confirmed as such by x-ray study but without operation, and 32 were proved at the operating table. In this group, 39 patients (24.1 per cent of the group) yielded sterile cultures; 24 patients (14.8 per cent of the group) yielded contaminated cultures, and 99 patients (61.1 per cent of the group) yielded positive cultures.

TABLE IV

GROUP 3. ORGANIC GASTROINTESTINAL DISEASE BUT WITHOUT BILIARY TRACT DISEASE

CASES	CULTURES			DIAGNOSIS CONFIRMED BY X-RAYS BUT WITHOUT OPERATION	DIAGNOSIS PROVED AT OPERATION
	STERILE	CONTAMINATED	POSITIVE		
Total No. (162)	39	24	99	42	32
Per cent	24.1	14.8	61.1		

Group 4 (Table V) consisted of 404 cases easily recognizable as gall bladder or duct disease. A number of them overlapped Group 7 with liver disease, and a number with Group 3 because in some instances there were both cholecystitis and peptic ulcer or upper right quadrant adhesions. The bacteriologic findings, however, have been listed but once. Of these 404 cases 108 were confirmed by x-ray study, but without operation, and 101 were proved at the operating table.

TABLE V

GROUP 4. GALL BLADDER OR DUCT DISEASE

CASES	CULTURES			DIAGNOSIS CONFIRMED BY X-RAYS BUT WITHOUT OPERATION	DIAGNOSIS PROVED AT OPERATION
	STERILE	CONTAMINATED	POSITIVE		
Total No. (404)	37	39	328	108	101
Per cent	9.2	9.7	81.1		

In this group, 37 patients (9.2 per cent of the group) yielded sterile cultures; 39 patients (9.7 per cent of the group) yielded contaminated cultures, and 328 patients (81.1 per cent of the group) yielded positive cultures.

Group 5 (Table VI) consisted of 69 patients who showed continued morbidity after having undergone one or more operations on the gall bladder. Some of these patients also overlapped Group 7, having liver disease. In this group,

TABLE VI

GROUP 5. CONTINUED MORBIDITY AFTER ONE OR MORE OPERATIONS ON GALL BLADDER

CASES	CULTURES		
	STERILE	CONTAMINATED	POSITIVE
Total No. (69)	7	8	54
Per cent	10	11.6	78.4

7 patients (10 per cent of the group) yielded sterile cultures; 8 patients (11.6 per cent of the group) yielded contaminated cultures, and 54 patients (78.4 per cent of the group) yielded positive cultures.

Group 7 (Table VII) consisted of 74 patients with liver or duct disease (hepatitis, cholangitis, cirrhosis, cancer—2 cases). In this group, 5 patients (6.8 per cent of the group) yielded sterile cultures; 6 patients (8.1 per cent of the group) yielded contaminated cultures, and 63 patients (86.1 per cent of the group) yielded positive cultures. It may be of interest that of the positive cultures B. coli was isolated 24 times, B. typhosus in 3 instances, B. pyocyaneus once, and the pyogenic cocci 46 times.

TABLE VII

GROUP 7. LIVER OR DUCT DISEASE

CASES	CULTURES		
	STERILE	CONTAMINATED	POSITIVE
Total No. (74)	5	6	63
Per cent	6.8	8.1	85.1

Comparing the analysis of these four groups, it becomes apparent that there is a distinctly higher percentage of positive cultures in Groups 4, 5, and 7 (patients with biliary tract disease) than in Group 3 (patients without biliary tract disease). Furthermore, both the sterile and contaminated groups in biliary tract disease are smaller than occurs in gastrointestinal disease, despite the fact that the same contaminating organisms, if contaminants, reach the duodenum in both groups.

I have already suggested that the interested reader of this article should carefully study the chapter in my monograph dealing with the bacteriologic

methods,* both from their clinical discussion and from the technical routine worked out by John A. Kolmer, because of the fact that so much greater detail is therein described, which obviously is not appropriate to this paper. In that chapter certain tables will indicate that Kolmer found that although various strains of streptococci and staphylococci were recovered in a large percentage of cases, nevertheless there was also a high incidence of the recovery of B. coli groups. Furthermore, of these B. coli groups several strains were definitely pathogenic to inoculated animals. On the death and autopsy of such animals the B. coli was recovered with impressive frequency from the portal blood and from the gall bladder bile, and furthermore, in a number of instances a gram-negative rod was demonstrated in the tissues of the gall bladder wall. Similar findings were also noted many times in rabbits inoculated with the pyogenic cocci. This may be significant.

However, in this paper it might be well to emphasize that the doctor or carefully trained technician should be on the alert, when taking cultures from the bile, to select through the glass observation window of the tube bile-stained rather than imbile stained floccules. The former are more apt to represent bacterial invasion of the biliary system; the latter, more apt to represent contamination brought down from the upper respiratory passages, mouth, or stomach. Also in the selection of such bile-stained floccules additional care should be exerted not to select the large, shaggy or slimy bile-stained floccules which under fresh spread appraisal indicate the characteristic microscopic picture of oleaginous degeneration so common to cystic duct catarrh and possibly cholesterosis.[22] The microscopic examination of such spreads is rarely as rich in bile-stained bacterial colonies as are the small fine, feathery or granular bile-stained floccules, often as minute as pinpoint size. Under microscopic appraisal such floccules more frequently reveal densely bile-stained colonies, in association with bile-stained pus cells and greater degrees of exfoliation of tall or short bile-stained columnar epithelium, in the characteristic fan-shaped or rosette clusters, which are supportive evidence of inflammatory disease of the mucosa of the gall bladder or ducts.

For some time I have been impressed with the frequency with which the B. coli groups have been recovered in bile drainages from patients who exhibit symptoms of what I have described as hepatic intestinal toxemia.[23] This would appear to suggest an impairment or loss of power of the bacterial function of the liver. This function seems to be a definite one and much of the published literature concerning it is already well known.[24]

Even in health there is evidence of a constant transfer of B. coli, the most common bacterial group in the human intestine, into the mesenteric veins, thence to the portal vein, and thence to the liver cells. These cells in health appear to have the power of destroying completely the germs brought to the liver (a bacteriolytic power) or of killing them and thus rendering them harmless for redistribution (a bactericidal power). Furthermore, there is general agreement that the B. coli group is chiefly resident in the ascending colon, in the cecum, and in the terminal loops of the ileum, and they become progressively less in

*See Reference 5-K, chapter 19, pp. 347-366.

numbers as one ascends the ileum to the duodenum. In other words, in health we are not accustomed to see B. coli invasion of the duodenum or the stomach, although obviously in a minority of cases such cultures can be obtained, but the explanation is not far to seek.

Therefore, in connection with our studies on the microscopy and culture of bile-stained floccules obtained on biliary drainage we have frequently noted the two following points: That in taking cultures from bile which has yielded B. coli, one group requires from eighteen to twenty-four hours for its demonstration in culture flasks, whereas in another group of patients an extraordinarily luxuriant growth of B. coli has occurred within two or three hours following the planting of the culture. This latter group, therefore, leads us to the suspicion that in such a patient both the bacteriolytic and bactericidal power in the liver cell is reduced or destroyed. The second point has been our observation that despite the recognition in our fresh spreads of large numbers of *bile-stained* bacterial colonies, morphologically approximating the appearance of B. coli groups, such bacteria have failed to be cultivable and have yielded us sterile cultures. In other words, such bacteria do not appear to be viable. This leads to a surmise that in such cases the bactericidal activity of the liver is still preserved but its larger function of complete bacteriolysis has been reduced or destroyed.

It seemed desirable, therefore, to analyze an eighth group (Table VIII), consisting of patients who exhibited symptoms of hepatic intestinal toxemia, in the attempt to learn whether the B. coli group would appear conspicuously frequent. This turned out to be a large group of 385 patients and necessarily overlapped Groups 3 to 7. B. coli was recovered in pure culture in 56.7 per cent, and B. coli in mixed culture together with streptococci or Staphylococcus aureus, B. pyocyaneus, B. lactis aerogenes and various enterococci was recovered in 23.1 per cent. Thus B. coli was recovered in 79.8 per cent of the entire group.

TABLE VIII

GROUP 8. HEPATIC INTESTINAL TOXEMIA

CASES	CULTURES				
	STERILE	CONTAMINATED	B. COLI PURE	B. COLI MIXED	PURE OR MIXED WITHOUT B. COLI
Total No. (385)	24	25	218	89	29
Per cent	6.2	6.5	56.7	23.1	7.5

The bacteria mentioned above, either in pure culture or mixed groups (but without B. coli), occurred in 7.5 per cent; contaminating cultures occurred in 6.5 per cent, and sterile cultures occurred in 6.2 per cent of this group.

To me this analysis appears to have significance because here the element of contamination from above the duodenal zone is reduced to its minimum, because the B. coli group can be cultivated from the duodenal zone in health with relative infrequency, and because such a high recovery of 79.8 per cent of B. coli in patients exhibiting symptoms of hepatic intestinal toxemia argues in favor

of a breakdown in the bactericidal function of the liver as an etiologic factor of importance.

As a result of the foregoing analyses I retain my conviction, despite the evident inaccuracies to which I and other investigators have called attention, that with careful technic in duodenobiliary drainage in regard to its bacteriologic aspects such a large number of positive cultures, as above defined, are obtained in biliary tract disease as to make it a matter of regret were we totally to disregard its diagnostic value and its therapeutic application.

Aside from the recognition of typhoid carriers of the hepatocholangitis type, and its usefulness in the detection of a B. pyocyaneus hepatitis in a case already reported twice,* its usefulness in the following case, selected from many similar ones in Group 7 of coccus infections of the liver and ducts, would alone justify its continued usage.

CASE No. 1920.—G. H. D., suffering from recurring attacks of jaundice with chills, fever, and sweats, was forty-nine years old when I first saw him in April, 1926. His past history was of importance. Although a healthy country-bred boy, with usual outdoor activities, as far back as he can remember he had bilious attacks, accompanied by headache, nausea, and vomiting. At twenty he had an attack of severe pain in the right hypochondrium not relieved by the usual measures, and accompanied by nausea. At twenty-three, because of supraorbital headache, blurred vision, and sleepiness after reading, his eyes were examined and compound hyperopic astigmatism was discovered and corrected with relief of these symptoms. His eyegrounds were negative. At thirty-eight he developed muscae volitantes which could not be seen with the ophthalmoscope. About this time he noticed tenderness in finger joints and recurrence of sciatica and lumbago, attacks of which he had suffered in childhood. He noticed a deep yellow stain appearing on his underclothes and pajamas under the armpits due to sweating. He was then mildly jaundiced. Over the next three years, upper abdominal symptoms of indigestion became more pronounced, and his weight gradually decreased from 190 to 160 pounds and the muscae volitantes became very troublesome. In 1922, at the age of forty-five, his leucocyte count was 13,000; therefore, his tonsils, which contained pus and streptococci, were removed. Four days later he had a severe attack of typical gall stone colic. X-ray examination showed considerable osteo-arthritis of the lumbosacral region, causing limited lateral rotation of the spine, but because the x-rays did not reveal stones, operation was deferred until October, 1923. His gall bladder was found to be small and thickened, and to contain 25 mulberry stones ranging from sand to pea size and one stone of olive size. The gall bladder and a diseased appendix were removed. The upper abdominal incision broke down and drained bile copiously although the cystic duct had been securely ligated. During this time he had severe nocturnal pains in the right chest and particularly under the right shoulder blade simulating severe pleurisy, together with drenching night sweats. The feces became clay colored, the urine dark, and he became temporarily jaundiced. The abdominal sinus drained until December when it superficially closed for five days. On the fifth day headache, general aching, severe chills, and fever ensued and the region of the wound became tender. It was reopened and two ounces of blood-stained bile was aspirated and cultured, yielding streptococci. The wound drained for three days and finally closed. Four months later headache, malaise, and tenderness over the upper right quadrant were followed by chills and fever (104° F.) for forty-eight hours which terminated after a profuse sweat. Clay stools and jaundice lasted for a week. This was the first of a series of similar attacks that recurred at frequent intervals. His stools were cultured and yielded a streptococcus. A vaccine was given but failed to influence the attacks. He then had his teeth and gums treated for pyorrhea and one infected molar was extracted. It is significant that on one occasion within twenty-four hours after removing tartar and cleaning the

*See Reference 5-K, page 501, and Reference 22, page 806.

teeth, he developed what was considered catarrhal jaundice, and a second attack within two days after extracting the infected molar tooth. Shortly after this time he came under my care.

His general appearance placed him in the liver group. His sclerae were markedly jaundiced, his skin was lemon yellow, his liver was considerably enlarged, and his spleen slightly so. The van den Bergh test gave a delayed direct reaction and the quantitative bilirubin of the blood was six times the normal. The leucocyte count was 9,200. He was placed in the hemolytic jaundice group. Biliary drainage gave evidence of hepatocholangitis with a suspicion of a residual stone. Culture yielded hemolytic streptococci only. His tonsils had already been removed, and cultures from his throat, his teeth, and gums failed to produce streptococci, so by inference his previous vaccine course might have whipped this focus without affecting his liver infection.

After it became evident that biliary therapeutic drainage with vaccines was not controlling his attacks, and the evidence of residual stone became more definite, he was referred for operation in April, 1927. The common duct was found partially strictured, behind which a small stone was found and removed. The stricture was dilated and T-tube drainage instituted. Cultures at the operating table and subsequently from the bile recovered by T-tube drainage yielded hemolytic streptococci in pure culture. T-tube drainage was continued for about seven weeks and on its removal drainage ceased, the wound healed, the sinus closed, and the patient has had no recurrence of chills, fever, or sweats accompanied by jaundice, although he has had three attacks (reported by telephone "follow-up" in January, 1932) of chills followed in twenty-four hours by "gouty" manifestations of the great toe.

After analyzing all of the data as presented in this paper we can see that there should be no great objection to considering that the B. coli, B. typhosus, and B. pyocyaneus are not contaminants but genuine infections. What argument exists, therefore, is concerned with the group of pyogenic cocci. In my opinion they should be considered in many cases to be transplants rather than contaminations, as illustrated in the foregoing case.

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LABORATORY METHODS IN THE TREATMENT OF PNEUMONIA*

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PNEUMONIA from a bacteriologic and immunologic viewpoint has been a development of the last fifty years. It was not until 1880 that Pasteur and Sternberg independently described the pneumococcus, and two years later that Koch recognized the tubercle bacillus. Frankel, in 1884, suggested that the pneumococcus was an important etiologic agent of pneumonia, and Weichselbaum, shortly after, provided conclusive evidence of this fact by obtaining pneumococci from a number of postmortem cultures. At about the same time that the pneumococcus was described, other bacteria, such as the staphylococcus, streptococcus, and Friedländer's bacillus were also being studied, and their morphology becoming known. These epochal discoveries were accompanied by the accumulation of important information regarding staining methods, culture media, animal pathogenicity, et cetera. In 1910, Neufeldt and Händel discovered that although the various strains of pneumococci were similar in morphology, cultural characteristics, sugar reactions, and bile-solubility, they were immunologically different. These investigators laid the foundation for a biologic classification of the pneumococci which was constructed by Dochez and Gillespie when they separated the pneumococci into Types I, II, and III, and Group IV.

Since the recognition of the immunologic types, the most important progress that has been made has been the development of specific treatment. However, there has been a great deal accomplished also in the bacteriologic and chemical studies of the pneumococcus. The organism itself has been divided into its various chemical fractions. The toxic and immune substances produced by these bacteria have been the subject of numerous investigations. The most recent noteworthy advances have been in the improvement of methods already known; of these, modifications of sputum typing and blood culture methods which give more rapid and more accurate results seem to have the greatest practical significance.

Upon the knowledge gained through these modern methods of bacteriology and immunology, a sound etiologic diagnosis of pneumonia has been established which changes our whole conception of this disease. The mere designation of

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pneumonia as lobar, broncho, or lobular, has great limitations in its aid to treatment, prognosis, and even to a general understanding of the disease.

The importance of distinguishing between a case of pneumococcus pneumonia and one of tuberculous pneumonia is generally recognized, today. Nevertheless, such a necessary differentiation cannot always be made by purely clinical means, but usually can be definitely made with the aid of laboratory methods. Neither can such diseases as hemolytic streptococcus pneumonia and Friedländer's bacillus pneumonia be differentiated by clinical methods, and here, again, laboratory procedures must be employed. Still the prognosis and attempts at specific treatment in these diseases are entirely dependent upon this etiologic diagnosis.

A complete bacteriologic study of each case of pneumonia, including examination and typing of the sputum, culturing of the blood, and, when indicated, culturing of the spinal and chest fluids, is indispensable for a thorough understanding of that case. At Bellevue Hospital, since 1920, a number of routine bacteriologic procedures have been carried out on all cases of pulmonary disease as part of an investigative study, but more and more have we become convinced of the practical significance of many of these procedures. At the present time our routine is as follows:

1. As soon after admission as the diagnosis of pulmonary disease is made, a sputum specimen is obtained. If the patient is not expectorating, a throat swab is procured. The specimens obtained from patients with pneumonia, or suspected of pneumonia, are sent to the laboratory for examination and typing. Sputum procured from patients with chronic pulmonary disease is examined and studied microscopically by the Ziehl-Neelsen and dark-field methods.

2. At the same time that the sputum or swab is obtained, cultures are made from the blood of all patients with pneumonia, or suspected pneumonia.

3. When the first sputum obtained from pneumonia patients has not shown a predominance of Type I or Type II pneumococcus, a second specimen is procured in order to confirm the initial findings. If sputum specimens of tuberculous patients give negative findings, other specimens are obtained, at regular intervals, for microscopic study.

4. When initial blood cultures taken on pneumonia patients are positive, they are repeated daily until the procedure is no longer indicated; when negative, they are repeated every second day until the toxemia subsides.

5. Spinal fluids, chest fluids, joint fluids, and pus obtained from infected foci are obtained whenever possible and sent to the laboratory for bacteriologic study.

6. Exudate is occasionally obtained by lung puncture and then only when the sputum or swab gives ambiguous or unsatisfactory results.

DIAGNOSIS OF PNEUMONIA

It has already been pointed out that the real value of the various laboratory procedures lies in the fact that they supply the information upon which depends the diagnosis, not only of the condition itself, but also of its complications. In other words, the etiologic diagnosis is based upon the laboratory findings, just as, in turn, the treatment is based upon the diagnosis.

Pneumonia, like many other diseases, has been classified according to a number of different systems; the two most important being the anatomic and the etiologic. In the anatomic classification, pneumonia is divided into lobar, broncho, and lobular pneumonia. This is still the most commonly accepted clinical classification, although it is not nearly as exact, and certainly not as significant, as the etiologic one. Cole has suggested the division of pneumonia into acute lobar pneumonia, primary atypical pneumonia, and secondary atypical pneumonia, determined primarily by the character of the onset and the course of the disease. The value of the anatomic classification and that of Cole is that through clinical findings they approximate the true etiologic diagnosis.

The etiologic classification of pneumonia is as follows:

1. Pneumococcus pneumonia.
   - A. Type I.
   - B. Type II.
   - C. Type III.
   - D. Group IV.
2. Hemolytic streptococcus pneumonia.
3. Staphylococcus pneumonia.
4. Friedländer's bacillus pneumonia.
5. Bacillus influenza pneumonia.
6. Mixed infections.
7. Infections of unknown etiology.

Clinically, it is impossible to differentiate the various etiologic varieties of pneumonia. Most of the pneumococcus pneumonias cause a lobar consolidation and run a typical course, but any one of the other kinds may follow the same course and show the same physical findings. On the other hand, the miscellaneous varieties (hemolytic streptococcus, Friedländer's bacillus, etc.) usually run an atypical course and have the signs of bronchopneumonia, but they may vary also. Furthermore, there is a great deal of overlapping of the lobar, broncho and lobular forms, and, likewise, it is not always easy to decide whether the course is typical or atypical. The etiologic classification, which is based on the infecting organism, is the only one that is clearly defined.

The incidence of the various etiologic types of pneumonia in a series of cases at Bellevue Hospital is shown in Table I. Over 95 per cent of the cases were caused by the pneumococcus, which emphasizes the importance of this organism in the etiology of this disease.

It is interesting to compare Table I with Table II, which shows a series of pneumonia cases in children (under twelve years of age) distributed according to the infecting organism.

In Bellevue Hospital, since 1920, all of the pneumococcus pneumonias in adults have been classified according to their immunologic types, and the results may be seen in Table III. A corresponding series in children, over a two-year period, is tabulated in Table IV.

Recently, Cooper, Edwards, and Rosenstein reported their work on the separation of the Group IV pneumococci into a number of fixed types. The

TABLE I

BACTERIOLOGIC CLASSIFICATION OF 2,000 CASES OF PNEUMONIA, BELLEVUE HOSPITAL, 1920-1925*

BACTERIA	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Pneumococcus	1,913	95.65
Hemolytic streptococcus	76	3.8
Friedländer's bacillus	8	0.4
Influenza bacillus	1	0.05
Staphylococcus aureus	2	0.1
Total	2,000	

*Cecil, R. L., Baldwin, H. S., and Larsen, N. P.: Lobar Pneumonia, Arch. Int. Med. **40**: 253-280, 1927.

TABLE II

BACTERIOLOGIC CLASSIFICATION OF 329 CASES OF PNEUMONIA IN CHILDREN, BELLEVUE HOSPITAL, 1928-1930

BACTERIA	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Pneumococcus	308	93.6
Streptococcus viridans	13	4.0
Hemolytic streptococcus	3	0.9
Staphylococcus	5	1.5
Total	329	

TABLE III

INCIDENCE OF PNEUMOCOCCUS TYPES IN LOBAR PNEUMONIA OF ADULTS TREATED IN BELLEVUE HOSPITAL, 1920-1930*

PNEUMOCOCCUS	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Type I	1,131	30.9
Type II	850	23.2
Type III	434	11.9
Group IV	1,247	34.1
Total	3,662	

*This series does not include cases admitted during the season of 1925-1926.

TABLE IV

INCIDENCE OF PNEUMOCOCCUS TYPES IN LOBAR PNEUMONIA OF CHILDREN TREATED IN BELLEVUE HOSPITAL, 1928-1930

PNEUMOCOCCUS	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Type I	32	10.4
Type II	9	2.9
Type III	10	3.2
Group IV	257	83.4
Total	308	

incidence of these new types at Bellevue Hospital, during the year 1928-1929, in both children and adults, can be seen in Table V. The prevalence of certain of the new types is particularly noted in the children's series.

The importance of an etiologic and type diagnosis in pneumonia cannot be too strongly stressed. Such a diagnosis is really essential to the administration of specific treatment on a rational basis. Furthermore, by knowing what is the infecting organism, the doctor has a key to probable complications, and more definite data for his prognosis, in short, he is equipped with a much better general scientific understanding of the case.

TABLE V

INCIDENCE OF TYPES IN PNEUMONIA, BELLEVUE HOSPITAL
Adult Cases, Nov. 1, 1928 to May 30, 1929
Children's Cases, Nov. 1, 1928 to April 30, 1929

PNEUMOCOCCUS	ADULTS		CHILDREN	
	NUMBER OF CASES	PERCENTAGE OF INCIDENCE	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Type I	75	18.1	14	9.5
Type II	140	33.7	3	2.0
Type III	31	7.5	3	2.0
Type IV	17	4.1	4	2.7
Type V	32	7.7	9	6.1
Type VI	7	1.7	19	12.9
Type VII	16	3.9	5	3.4
Type VIII	3	0.7	1	0.7
Type IX	6	1.4	6	4.1
Types X, XI, XII, XIII	8	1.9	3	2.0
Unclassified	72	17.3	73	49.7
No Pneumococci	8	1.9	7	4.8
Total	415		147	

EXAMINATION OF THE SPUTUM

In all types of pulmonary disease, the sputum is the image of the process in the lung, but a careful examination of this image is required and the findings must be accurately interpreted to be of any value. In a small percentage of pneumonia patients no sputum is procurable. In such cases a throat swab may give the same results. From our viewpoint, the bacteriologic examination of the sputum is the most important, but the histologic findings are also significant, particularly in certain forms of pulmonary disease. If the sputum of patients with diseases of the lung is carefully and repeatedly examined there are sur-

prisingly few instances in which it does not disclose the infecting organism and give a good clue to the process existing in the lung.

A great deal of care should be taken in obtaining specimens of sputum for examination. The essential points are to obtain a specimen from the deeper air-passages, free from saliva, and to collect it in a sterile container such as can be easily handled, and will be convenient for careful gross examination. In a hospital, it is most satisfactory to collect the sputum in a sterile Petri dish. The patient is directed to use the dish only when the sputum is expectorated from the lung. If the patient has not raised the required sputum of his own accord, within one or two hours, he is asked to cough deeply once or twice, while lying on his normal side, and if this is not effective, a throat swab is procured. The specimen should be sent to the laboratory as soon after collection as possible. In case of delay, it should be kept in an ice box or refrigerator until it can be examined.

The gross appearance of the sputum is extremely important, and frequently is an invaluable factor in the clinical findings. Examination should be made with a consideration of the following:

1. Amount.
2. Consistency: watery, tenacious, mucoid, mucopurulent, purulent, nummular, bloody.
3. Color: colorless, rusty (prune-juice), bloody (fresh blood), yellow, green, brown, black, white, bile-colored.
4. Odor: odorless, musty, sweetish, foul.
5. Particulate material: caseous particles, foreign bodies, casts, pneumoliths, broncholiths, Curschmann's spirals.

A pneumococcus pneumonia usually produces a characteristic rusty (prune-juice), tenacious sputum. Early in the disease, it may be frankly bloody and in the later stages, as resolution progresses, it may become mucopurulent, or occasionally purulent. It is not possible to distinguish the various types of pneumonia by the gross appearance of the sputum alone. In streptococcus pneumonia the sputum is characteristically greenish, and purulent; in staphylococcus pneumonia it is yellowish and purulent; in Friedländer's bacillus pneumonia it is extremely tenacious.

Microscopic examination of direct films of the sputum is an important procedure and should always be included in the laboratory routine. Direct films are best made in the following way: A small particle of the sputum to be examined is placed on the right-hand third of a clean slide which is held in the left hand, and another slide is placed over the sputum. The slides are then pulled apart, leaving a film of sputum on the opposing surfaces. Immediately, both slides are warmed, carefully, over a Bunsen flame, and, at the same time, the surfaces covered with sputum are scraped together until the slides have the appearance of ground glass. In this manner an even film is insured.

The films are stained by the Gram or Ziehl-Neelsen method. The microscopic study of sputum supplies information regarding the cellular structure of the specimen and thereby gives some indication of its source. Of chief significance is the information which these studies give regarding the pre-

dominance and nature of the organisms present. Acid-fast organisms are readily distinguished by the Ziehl-Neelsen method. The pneumococci and other pyogenic organisms are best shown by the Gram stain. By the latter method the capsules of the pneumococcus are occasionally detected. However, when a more careful examination of the capsule is required, Hiss's copper sulphate method is an excellent one to employ. In some cases the Type III pneumococcus is definitely identified because of its characteristically large capsules. Gentian violet stain is effective for the demonstration of fusospirochetal organisms and is recommended for the examination of sputum from patients with lung abscess and bronchiectasis.

SPUTUM TYPING

The purpose of typing the sputum is to separate the pneumococci into their various immunologic groups, in order to complete the etiologic diagnosis. This type diagnosis has value in prognosticating, but is even more valuable in outlining specific therapy. Pneumococcus immune serum is type specific. It is required of serum therapy that the preparation used should contain antibodies against the particular type causing the infection, and it is equally important that it be administered as early as possible in the course of the disease. With these facts in mind, it is readily understood that the essentials of typing are: accuracy, rapidity, and efficiency.

In recent years, at Bellevue Hospital, a procedure has been adopted in which certain features of a number of accepted methods of typing are employed. This procedure is based, primarily, on the use of the mouse for culturing the pneumococcus and separating it from the various normal mouth organisms, and also upon the utilization of the agglutination and precipitation phenomena which occur when the specific immune serum is added to a growth of pneumococci. This combination method is carried out in the following manner:

The sputum to be examined is collected in a sterile Petri dish and sent to the laboratory. The gross appearance is carefully noted and described, and the necessary films are prepared.

The cover of the Petri dish is removed and inverted. The sputum is washed in 2 to 5 c.c. of sterile physiologic saline solution, in order to free it from saliva and mouth contaminants. A small portion of the sputum, about 1 cm. in diameter, is selected and transferred to the Petri dish cover, into which 2 c.c. of the physiologic saline has been poured. The sputum is then emulsified, first by whipping it with a small applicator, and then by forcing it through a sterile 2 c.c. Luer syringe, several times. When the mixture is thoroughly emulsified, from 0.5 to 1 c.c. is retained in the syringe, for mouse inoculation. A white mouse, of good breed, of either sex, weighing from 18 to 22 grams, is used. The injection is made intraperitoneally, preferably directing the needle through the muscles of the thigh and thence to the abdomen, the site of the injection having been carefully sterilized with 95 per cent alcohol.

When sputum is not available, a throat swab is procured by streaking the posterior pharynx with a cotton applicator several times or until the patient coughs. The swab is cultured for two hours at 37° C. in 3 c.c. of beef-heart

infusion broth, P_H 7.8, to which 0.15 c.c. of defibrinated blood has been added. At the end of this time 1 c.c. of the culture is injected intraperitoneally into a mouse, and the pneumococcus typing is carried out in the same manner as when sputum is used for the examination.

If the specimen of sputum is sufficient in amount and of the characteristic pneumonia type, the Krumwiede method of typing is carried out. This method is here described.

KRUMWIEDE RAPID PNEUMOCOCCUS TYPING

A portion of sputum (5 to 10 c.c.) is placed in a 15 c.c. centrifuge tube. The tube is set in boiling water for three to five minutes to coagulate the albuminous portion and to free the soluble substance. If the quantity of clear, supernatant fluid which contains the soluble substance is sufficient to complete a precipitation test with the type specific antipneumococcus serum, it is desirable to use it in its undiluted form. In case the quantity is not sufficient (is less than 0.6 c.c.), it is necessary to add a sufficient amount of physiologic saline (not more than 0.5 c.c.) in order to complete the test. If too much saline is added, the soluble substance which is produced in variable quantities by the different types of pneumococci may be so diluted as to prevent a positive reaction. If saline is added, the tube is again placed in boiling water, and is stirred occasionally, for two minutes, in order to completely extract the soluble substance. The tube is then centrifuged at a high rate for ten to fifteen minutes. The supernatant fluid is removed from the tube and is layered, in amounts of about 0.2 c.c., on equal volumes of undiluted diagnostic serums contained in small culture tubes. A test is positive when a precipitation ring forms between the two layers. The tubes used must be clean and perfectly transparent, because the precipitation ring is difficult to distinguish. In order to confirm the results of the ring test, the tube is shaken and incubated for fifteen minutes at 37° C. At the end of this time, in case of a positive finding, a fine white flocculation is noted. The success of this method depends upon (a) obtaining from the patient an ample specimen of pneumonia sputum expectorated from the deeper air passages and (b) the delicacy of the technic employed. In competent hands, this method gives very accurate results. The principle disadvantage is that it can be used in only about 25 per cent of pneumonia cases. When this method is impracticable, or gives negative results, the Sabin rapid method of typing is carried out.

SLIDE AGGLUTINATION TYPING OF SABIN

"From three to four hours after the injection of the mouse, some of the peritoneal fluid is obtained by puncture with a glass capillary. A glass slide is marked off into four parts, and a minute drop of the peritoneal fluid is expelled on each one of the four partitions. The first is smeared with saline for control, and the others with a loopful of 1:10 dilution of Type I and of Type II, and a 1:5 dilution of Type III diagnostic serums, respectively. These dilutions of serum are chosen largely to eliminate group agglutinins. The smears are made thin, allowed to dry, and fixed by passing the slide through a

flame; they are then stained from twenty to thirty seconds with a fuchsin solution (10 c.c. saturated alcoholic solution of basic fuchsin plus 90 c.c. of water) or any other available stain. The stain is washed off in water or 20 per cent copper sulphate solution, and the smears are examined with the oil immersion lens. If a specific agglutination reaction is observed in one of the smears with diagnostic serum, the organism is of that type. If no reaction occurs in any of the smears, and numerous pneumococci are clearly seen, a diagnosis of Group IV is suggested. When it is desired to know whether the organism is one of the fixed types of Group IV (especially those for which concentrated antiserums are available), a similar procedure is carried out with the corresponding diagnostic serums. Bacteria in the sputum which are not pneumococci as well as avirulent forms of pneumococci may occur in clumps in the peritoneal exudate; but these differ in appearance from those produced by specific agglutination; they can be distinguished further by their occurrence in the saline control smear as well. Unless a fresh sample of sputum is used, many of the organisms will have undergone autolysis, and therefore more time must be allowed for growth. Since the mouse is not killed, another typing can be done if the first one should show insufficient organisms, and after death of the mouse, the type may be confirmed. In the case of Type III, sufficient organisms are usually present even two hours after injection. The appearance of the specific reaction with Type III differs somewhat from that obtained with other types of pneumococci, primarily on account of the larger size of the capsule; the organisms are further apart in the agglutinated clumps which occur in mucoid strands."

If a positive finding has not been obtained by either the Krumwiede or the Sabin method of typing, after the mouse has died, the macroscopic agglutination and precipitation tests are carried out on the peritoneal exudate in the following manner:

The peritoneal cavity is opened with sterile precautions, and a culture is made by streaking some of the exudate on a blood agar plate by means of a platinum loop. At the same time, films are made from the exudate. Next, the peritoneal exudate is washed with 5 c.c. of sterile saline solution by means of a sterilized glass dropper. The washings are placed in a sterile centrifuge tube. Before the body of the mouse is discarded, the thorax is opened and a blood agar plate is streaked with a loopful of the heart's blood.

Typing is continued by centrifuging the peritoneal washings, first, at a low speed, in order to throw down the cells and fibrin, and then, after transferring the supernatant fluid to another sterile centrifuge tube, at a high speed, in order to separate the organisms. After this procedure, the supernatant fluid is transferred to a tube to be used for the precipitin test. The bacterial sediment is resuspended in about to 2 to 3 c.c. of normal saline solution, a sufficient quantity being used to give the appearance of an eighteen-hour broth culture of pneumococcus. This bacterial suspension is used for the agglutination test.

For these tests small culture tubes (3 by ⅜) are arranged in two rows in racks for placing in the water-bath. One row of tubes is used for the agglutination test and the other, for the precipitation test. For the former, 0.2

c.c. of a 1:10 dilution of each diagnostic serum is transferred to the tubes with a 1 c.c. pipette. For the latter, the same procedure is carried out, using undiluted diagnostic serum.

The bacterial suspension is used for the agglutination test, and the centrifuged supernatant fluid is used for the precipitation test; for both, 0.2 c.c. amounts are added to the tubes already arranged. The tubes are then incubated for two hours in a water-bath, at 37° C., or for two hours at 56° C. At the end of this time the tubes are carefully examined for clumping and flocculation, indicative of the specific type.

Confirmation of Type.—The type obtained by any of the aforesaid methods is confirmed by typing the pneumococci cultured from the heart's blood of the mouse. After streaking the blood agar plate with the heart's blood, at the time of the mouse autopsy, the plate is incubated for eighteen to twenty-four hours, at 37° C. At the end of this time a discrete colony of pneumococci is transferred, with a platinum loop, to 9 c.c. of beef-heart infusion broth containing 5 per cent of defibrinated blood. This culture is incubated for twelve to twenty-four hours and then 0.5 c.c. of it is added to 9 c.c. of plain broth, and, again, incubated for the same length of time. This broth culture of pneumococci is typed by the agglutination method outlined above, and, at the same time, the organisms are tested for bile solubility by adding 0.3 c.c. of the culture to 0.1 c.c. of whole ox bile. A solution of pure sodium desoxycholate, one part to 500 parts of culture, may be used instead of the whole bile. This method of typing gives exceedingly accurate results which are valuable in confirming those obtained by the more rapid methods.

BLOOD CULTURES IN PNEUMONIA

The taking of blood cultures in pneumonia is an important diagnostic and prognostic procedure. The etiologic diagnosis is usually first made by the sputum typing. However, finding the same organism in the blood is important confirmatory evidence. Perhaps, even more significant, from a diagnostic point of view, is the value of this procedure in recognizing complications of pneumonia. Of these complications, septicemia is one of the most frequent. Many of the others, such as meningitis, endocarditis, and pericarditis occur as sequelae of the septic condition rather than of the pneumonia itself. In prognosis, the determination of the degree of septicemia is more elucidating than the mere discovery of its presence.

The mortality in pneumonia patients whose blood contains more than 100 organisms per cubic centimeter is practically 100 per cent. On the other hand, the outlook is by no means hopeless in the presence of only a few organisms, particularly when found early in the disease.

Blood cultures are best made at the patient's bedside. Three cubic centimeters of blood is removed, under sterile precautions, with a 5 c.c. syringe. Of this, 1 c.c. is inoculated into 9 c.c. of plain broth and the remaining 2 c.c. is used for two agar pour plates. These cultures are then sent to the laboratory and incubated, at 37° C., for twenty-four hours. At the end of this time, the colonies on each plate are counted and the broth is typed by the agglutination method, as described above, care being taken to prevent shaking of the tube

before the suspension is removed. Negative cultures are allowed to remain in the incubator for four days before the final report is given, and the tubes and plates are discarded.

CHEST FLUID, SPINAL FLUID, AND JOINT FLUID CULTURES IN PNEUMONIA

Empyema, meningitis, and septic arthritis are not rare complications of pneumonia, particularly following pneumococcemia. Whenever fluid is removed from the chest, spinal canal, or joint, it should be submitted to careful bacteriologic study. The appearance of these fluids is usually sufficient to diagnose the particular complication. However, knowing the number and variety of organisms present gives additional significant information, particularly in confirming the results of the original typing.

The bacteriologic study of the fluids should be complete. It is well, first of all, to make films and stain them by the Gram method. The fluid should be streaked on a blood agar plate and 0.5 to 1 c.c. injected into a mouse. The pneumococci are typed from discrete colonies on the plate or from the mouse exudate, in the manner described under sputum typing. When pneumococci are present in large numbers in any of the body fluids they may be typed directly by the Sabin slide agglutination method.

COMMENT

Perhaps the greatest advance in the practical laboratory procedure in pneumonia within the last few years has been in the modification of bacteriologic methods to give more accurate and more rapid results. The use of the Sabin rapid method of typing makes it possible to obtain an accurate type diagnosis on the day the patient is first seen (before its adoption, there was always a delay of from eighteen to twenty-four hours). The chief practical advantage of this rapid typing is that a type diagnosis can be made as a preliminary to the consideration of specific therapy.

In the early days of serum therapy, one of the major criticisms was that a large number of patients were subjected to the inconvenience and expense of serum injections and later were shown to have a type of infection that was not compatible with the serum administered. In addition to the improvements in typing, there have also been improvements in blood culture methods. For instance, it has been found a distinct advantage to withdraw a small amount of blood, such as 3 c.c., for which a small needle and a small syringe are adequate. With skillful technic this operation causes practically no discomfort to the patient, and if repeated at regular intervals becomes one of the most valuable aids in prognosis.

Shortly after Sabin described his rapid method of typing, other investigators reported modifications which in their own hands gave comparable results. Armstrong suggests the examination of an unmixed and unstained mixture of mouse exudate and diagnostic serum in order to observe the agglutination of the organisms. Calder uses the hanging-drop method and a vibrating machine which he has devised in order to obtain definite and rapid agglutination. Each of these three methods is dependent upon finding a sufficient num-

ber of organisms in the peritoneal exudate. Hence, they are all unsatisfactory in certain cases. The most important factor in the operation is experience with the method used.

During the past few years, pneumonia has been the subject of extensive investigation, but most of the discoveries have not yet reached the stage of practical significance. Perhaps the most important advance toward the solution of this problem has been the chemical fractionation of the pneumococcus. This information has recently been applied by Avery and Dubos to the development of an enzyme which splits the carbohydrate fraction of the Type III pneumococcus, and by so doing destroys the toxicity of this organism. Already these scientists have been able to protect mice against the Type III pneumococcus infection, and it is probable that this agent will be of value in the treatment of pneumonia.

The artificial transmutation of types is another achievement of recent years. Griffith and Dawson have described methods of transforming pneumococci from one type to another, both in vivo and in vitro. These discoveries add a great deal to our knowledge of the pneumococcus and the infection which it produces. At present, however, we have no realization of their ultimate value.

The development of the modern treatment of pneumonia has passed through various stages. The first might be called the prebacteriologic era; the second, the pretyping; the third, the prerapid typing, and the fourth, that of the present day. Very few doctors are now practicing in the prebacteriologic era, but an isolated case occasionally comes to our notice. For example, a certain patient is known to have been treated as having lobar pneumonia, followed by delayed resolution, and after six weeks of care at home, surrounded by children, the consultant discovered numerous tubercle bacilli in the sputum. This unfortunate patient certainly was handled according to prebacteriologic methods.

The majority of pneumonia patients today are not given the benefit of typing, even when the advantages of type diagnosis are so striking, as shown by the following example: Two pneumonia patients with the same amount of lung involvement and the same degree of toxemia are seen late in their infection. Assume that one is a Type I, with a negative blood culture, and the other, a Type III, with a positive blood culture. The Type III patient will almost certainly die, the Type I has a good prognosis, and, if given the benefit of an efficient therapeutic agent, such as concentrated serum, will almost certainly recover.

In the up-to-date management of pneumonia, the sputum should be carefully examined and rapidly typed as a guide to prognosis, and to insure the prompt administration of serum in types in which it has been found efficacious. Blood cultures and other laboratory procedures should be instituted in order to recognize early the presence of septicemia and other complications. In short, it is evident that the modern treatment of pneumonia is dependent upon the utilization of the latest bacteriologic and immunologic methods.

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(Symposium to be continued in April issue)

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EDITORIAL

The Symposium

A symposium on clinical bacteriology is of particular significance and value at the present time when the news dispatches in featuring the discoveries in the field of medical bacteriology are usually guilty of a hyperbole. The press when using such descriptive statements as: "The most important step in medical bacteriology since Pasteur"; "This discovery overthrows all of the well-founded tenets of this branch of the natural sciences"; ad nauseam, is only too frequently repeating the hopes of the worker rather than an evaluation of the data by the investigator's peers.

Such projected achievements as: "The sterilization of a city water supply by the occasional addition of a test tube full of a tiny destroyer of germs, the bacteriophage," may erase for a moment from the minds of the public health workers the worries of swimming pool control, but the statement: "The invisible virus of encephalitis is ever present among us as the visible hay bacillus," only causes consternation in the ranks. Again one reads: "The causative agent

of influenza is easily unmasked by a diet of hog's intestine," which is all the more confusing when the reader remembers an announcement of a short time before, "The agent of influenza masquerades as a pleomorphic streptococcus."

The effect of such bombastic circumlocutions, which ordinarily in due course of time prove to be plain rhetoric, on the public and the routine laboratory technician cannot be overestimated. The former visualizes the sudden conquest of all the afflictions of mankind while the latter only too often begins to doubt the value of the tried and true procedures and attaches a subterfuge for all uncertainties and failures.

Recent investigations on life cycles, mutation, dissociation, virus stages, sexual reproduction, filterable forms, etc., in bacteria are of extreme academic interest but their significance must be thoroughly understood and their influence on diagnostic technic carefully demonstrated before they are introduced into routine methods, lest pandemonium result. To insist on a demonstration of value is often extremely irritating to the exponent of a new theory and some accept to follow lest the exponent hurl the facile sentimentality, "The nature of your background and training incapacitates you for sympathy with modern and progressive thought," when as is usually the case, the poor worker only happens to be one of those unbiased readers who calmly surveying the evidence is astounded perhaps more by the easy saltations in the enthusiast's judgment and interpretation than in the bacterial form or phenomenon he is describing.

It is a pleasure on perusing the various contributions, so finely diversified, to find the cautious interpretations placed on the results of the separate procedures by the several writers, authorities in their own fields, yet the confidence in the results when the methods are carefully followed.

M. H. S.

Program of the International Congress on Asthma

At Mont-Dore, France

June 4 and 5, 1932

1. Evolution of Our Knowledge of Asthma—Fernand Bezancon.
2. Pathological Physiology of the Asthmatic Crisis—Abrami.
3. Anaphylaxis in Asthma—P. Vallery-Radot.
4. Nervous Factor in Asthma—Etienne Bernard.
5. The Liver and the Endocrine System in Asthma—Cordier.
6. Etiology and Pathology in the Treatment of Bacterial Asthma—Haibe.
7. The Role of the Nasal Ganglion in the Production of Asthma—Halphen.
8. Respiratory Equivalents of Asthma—Bourgeois.
9. Morbid Relationships of Asthma—Andre Jacquelin.
10. Etiology, Pathogenesis in Treatment of Infantile Asthma—Lesne.
11. Diagnostic Signs and Evolution of Infantile Asthma—Pehu.
12. Treatment of the Asthmatic Crisis—Joltrain.
13. Treatment of the Asthmatic Terraine—Lucien De Gennes.

14. Hydro-Mineral Treatment of Asthma—Villaret and Besancon.
15. Physiotherapy in Asthma—Biancani.
16. Surgical Treatment of Asthma—Leriche and Fontaine.
17. Mont-Dore and Asthma—J. Galup.

The above contributions are from among the leading French physicians interested in asthma. The following are on the program as representatives of foreign countries:

Professor Prausnitz (Germany).
Mac Dowall (England).
Clementino Fraga (Brazil).
Mariano Castex (Argentina).
Maranon (Spain).
Longcope (United States).
Storm Van Leeuwen (Holland).
Frugoni (Italy).
Danielopolu (Roumania).

Inquiries and communications should be directed to the Secretary General of The International Congress on Asthma, Dr. J. Galup, 19 Rue Auber, Paris, France.

Erratum

Through an error in make-up, abstract material that had been previously published was reprinted in the February issue.